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For many years artificial insemination has been the most widely used assisted reproductive technique (ART) in the horse breeding industry (Squires 2005), while other procedures based on the in vivo and in vitro production of equine embryos have emerged only in recent years.

Reasons for the delayed development of in vivo and in vitro production of equine embryos, compared to other domestic species, namely ruminants and pigs, include the scarce availability of abattoir ovaries and the lack of interest from horse breeders and registries. Moreover, in spite of the early success of equine oocyte in vitro maturation (Zhang et al. 1989), no further relevant research on the issue was reported in the horse for some time. Many efforts instead concentrated on conventional in vitro fertilization (IVF) but, unfortunately, the successes with IVF in other species never occurred with horses and only two foals were reported as born from IVF and both were derived from in vivo matured oocytes collected by ovum pick-up (OPU) from gonadotrophin-stimulated mares (Palmer et al. 1991; Bezard 1992).

The application of intracytoplasmatic sperm injection (ICSI) to the horse has overcome the barrier of inefficient IVF, resulting in the first pregnancy derived from an in vitro matured oocyte (Squires et al. 1996) which was successfully carried to term. In the following years, a few other scientific reports showed that it is possible to obtain pregnancies and live foals after collection of immature oocytes by Ovum Pick Up (OPU) followed by in vitro maturation, ICSI and immediate transfer (Cochran et al. 1998, McKinnon et al. 2000), or in vitro embryo culture (IVC) and non-surgical transfer of blastocyst stage embryos (Galli et al. 2002).

Today the most practical use of OPU or trans-abdominal follicle aspiration is to recover in vivo matured oocytes (Carnevale 2004) for oocyte transfer. It has been shown that the use of this procedure...
results in satisfactory pregnancy rates except for intrinsically compromised oocytes collected from geriatric mares (Carnevale and Ginther 1995, Carnevale et al. 2005).

OPU has been proven safe and repeatable in mares (Galli et al. 2002, Bogh et al. 2003, Vanderwall et al. 2006). When used in combination with in vitro maturation and ICSI, OPU has the considerable advantage of not requiring any hormonal stimulation of the donor and this aspect is of particular importance in mares because superovulation still gives unsatisfactory and inconsistent results. Another advantage of using OPU-ICSI-IVC is the possibility to widen the choice of stallions to be used, including those with poor sperm motility and reproductive performance in vivo (Lazzari et al. 2002). Finally an important application of OPU-ICSI-IVC is for rescuing the fertility of aged donors. It is well known (Carnevale and Ginther 1995, Hemberg et al. 2004, Madill 2002) that mare fertility decreases after 12-13 years of age. In fact, mature mares can be more easily affected by many reproductive problems that can arise from abnormalities of the external genitalia, altered neuro-endocrine system functionality, ovarian or uterine pathologies or simply any anomaly related to ageing. It has also been reported (Hurtgen 2006) that endometritis has more deleterious effects on aged mares than on young animals.

This manuscript will focus on the clinical application of OPU-ICSI-IVC in the horse breeding industry and will describe the results obtained in Italy by commercially using this technique over a five year period. During the period 2004-2008, data were collected from 78 commercial mares of 3-24 years of age that were elected to produce offspring by using OPU, ICSI and IVC. Frozen-thawed semen from 67 stallions of varying different quality and fertility was used. During the breeding season mares were subjected to OPU in diestrus in the absence of a dominant follicle if at all possible. Oocytes were collected by transvaginal ultrasound-guided follicular aspiration (Ovum Pick Up) from donor mares as previously described (Galli et al. 2002). Briefly all the ovarian follicles ranging from 0.5 to 4 cm diameter were aspirated using a 12G coaxial double lumen needle connected to an aspiration pump. The recovered oocytes were then in vitro matured by culturing them in TCM199 or DMEM-F12 based medium (Galli et al. 2007). The oocytes reaching the metaphase II stage were in vitro fertilized by ICSI and in vitro cultured up to the blastocyst stage. The blastocysts were either transferred fresh or frozen in 10% glycerol and subsequently stored in liquid nitrogen. Embryos were frozen at day 6, 7, 8 or 9 (day 0 is the day of ICSI) in relation to the time when the blastocyst stage was achieved. Embryos were transferred non-surgically to recipient mares 4 to 6 days (preferably 5 days) after spontaneous ovulation.

Forty-three of the 78 mares recruited in this program were reproductively sound while the other 35 were aged mares or showed various reproductive disorders (i.e.: degenerative endometriosis with history of large post-insemination fluid accumulation, non repairable cervical laceration, genetic aplasia of a uterine horn, etc) that partially or totally limited their ability to produce offspring, even by using conventional embryo transfer.

One hundred and forty-five oocyte collections were performed by using ovum pick up at various stages of oestrus cycle and 2453 ovarian follicles collected transvaginally by ultrasound guided follicular aspiration.
The total number of oocytes collected was 1553 (63.31%) of which 1003 (64.58%) reached metaphase II, following in vitro maturation. The matured oocytes were fertilized by ICSI and gave rise to 640 (63.80%) cleaved embryos that were subsequently cultured in vitro. A total of 103 blastocysts (0.71 per OPU) were obtained corresponding to a 10.26 or 16.09% development rate calculated on the number of matured oocytes or on the cleaved embryos, respectively. All the 103 blastocysts were frozen, 84 of them were non-surgically transferred afterwards into recipient mares and 49 (58.33%) yielded a pregnancy. Fourteen foals were born, and three pregnancies are still ongoing.

In conclusion, Ovum Pick Up, Intracytoplasmatic Sperm Injection and Embryo Culture are techniques that can be successfully used in the equine industry to obtain embryos from mares that have partially or totally lost their ability to produce offspring and in many cases represent the only option available. The same apply to stallions with low fertility in vivo or cases when limited amount of frozen semen is available and it is not sufficient to perform artificial insemination. Embryos can also be produced outside the breeding season and frozen for later transfer, thus making this technique very flexible and versatile in a clinical setting. The pregnancy rate obtained in this retrospective study after non-surgical transfer of ICSI cryopreserved embryos is similar to those reported when transferring fresh or cooled embryos produced by conventional embryo transfer and shows that the use of OPU-ICSI-IVC offers a real opportunity for breeding valuable stock in equine practice.

<table>
<thead>
<tr>
<th>N° of donors</th>
<th>N° of OPU</th>
<th>N° of follicles</th>
<th>N° of oocytes (recovery rate)</th>
<th>N° of injected (%MII)</th>
<th>Cleaved (Cl/Inj)</th>
<th>N° of Blastocysts (per OPU)</th>
<th>Blastocysts/Injected</th>
<th>Blastocysts/Cleaved</th>
</tr>
</thead>
<tbody>
<tr>
<td>78</td>
<td>145</td>
<td>2453</td>
<td>1553 (63.31)</td>
<td>1003 (64.58)</td>
<td>640 (63.80)</td>
<td>103 (0.71)</td>
<td>10.26</td>
<td>16.06</td>
</tr>
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References


