Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 70 (2008) 441–444
Theriogenology
www.theriojournal.com

When should a mare go for assisted reproduction?

M.A. Coutinho da Silva *

Department of Clinical Sciences, Cornell University, Ithaca, NY 14853, United States

Abstract

The use of assisted reproductive techniques (ART) has helped owners to produce offspring from valuable mares that were considered infertile using standard breeding techniques. Before referring a mare for an ART, the practitioner should be able to identify the underlying cause of subfertility of the mare. The objective of this review is to provide information regarding embryo transfer, oocyte transfer and intracytoplasmic sperm injection, the three most common ART used in equine practice. Knowing the complexity as well as the risks of these techniques, enables practitioners to refer a subfertile mare to the least complex and most appropriate and successful ART that can overcome specific causes of infertility.

Keywords: Assisted reproduction; Oocyte; ICSI; Embryo; Equine

1. Introduction

Every year, many mares fail to become pregnant, despite several attempts using semen from a fertile stallion and good management practices. These mares are considered subfertile and represent a substantial economic and genetic loss to the horse industry. Depending on the cause of subfertility and breed registry restrictions, most subfertile mares can still produce offspring through the use of assisted reproductive techniques (ART).

Over the last two decades, ART have progressed from AI and embryo transfer (ET) to oocyte transfer (OT), intracytoplasmic sperm injection (ICSI), and nuclear transfer (cloning). Advances in ART in the horse have been driven by the desire to: (1) establish pregnancies in mares that would otherwise be infertile, (2) develop means for rapid multiplication of certain genetic lines, (3) study the biology of oogenesis, fertilization and early embryo development, and (4) provide material for other technological advances, such as micromanipulation or genetic engineering [1].

* Tel.: +1 607 253 4189.
E-mail address: mac253@cornell.edu.

0093-691X/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2008.05.039

There is substantial variation among specific ART in complexity, cost, indication, and success rates. The cost of the procedure is directly related to its complexity and personnel and laboratory costs involved. Procedures requiring expensive equipment and highly trained professionals, such as ICSI, are more costly. Ironically, these same expensive procedures usually have lower efficiency compared to ET or OT (Table 1). However, each ART was developed to circumvent specific problems that affect fertility; therefore, diagnosis of the underlying cause(s) of subfertility is crucial for an appropriate referral of a mare for an ART.

In order to help practitioners decide on which ART is appropriate for a particular subfertile mare, this article will briefly discuss three important techniques (ET, OT and ICSI), focusing on the complexity, indication and risks associated with each procedure.

2. Embryo transfer (ET)

The first ET foal was produced by Oguri and Tsutsumi in 1974 [2]. Since then, the technique has been refined by increasing the care of the embryo during

Table 1
Complexity, cost, and success rate of assisted reproductive techniques used in equine practice

Procedure[a]	Complexity	Cost	Success rate (%)
ET	+	*X*	33–38[b]
OT	++	1.25–1.5*X*	23–27[b]
ICSI	+++	1.5–2.0*X*	13–18[c]

[a] ET – embryo transfer, OT – oocyte transfer, and ICSI – intracytoplasmic sperm injection.
[b] Success rate per cycle based on collection rate and pregnancy rate.
[c] Success rate per cycle based on blastocyst rate and pregnancy rate.

collection and handling, as well as perfecting the transfer technique. The standard method of embryo collection in the mare is a non-surgical transcervical uterine lavage [3,4]. A sterile catheter is inserted through the cervix, and the uterus is lavaged with embryo flush medium. The flush medium is allowed to flow back out the uterus and is passed through an embryo filter. Contents of the filter are poured into a search dish and examined.

Transfer of equine embryos can be performed by a surgical or a non-surgical transcervical approach. Today, nearly all embryos are transferred non-surgically into the uterus of synchronized recipients [5]. Non-surgical ET has generally been performed using: (1) a standard AI pipette, (2) a disposable plastic ''insemination gun'', or (3) a reusable stainless steel ''insemination gun'' [6]. Regardless of which instrument is used, an outer guard is generally placed over the transfer instrument to minimize contamination of the uterus. The embryo can be deposited in the uterine body or in one of the uterine horns.

The complexity of ET is relatively low compared to more advanced techniques, since embryos are transferred non-surgically into the uterus of recipients, allowing practitioners to perform ET on the farm. In that regard, ET can be easily performed by practitioners that are proficient in general equine reproductive management. Risks associated with the ET procedure are similar to those in any AI program and are associated with transrectal examinations, AI, and uterine lavage. The difficulty of the procedure lies in organization and coordination of the separate components that affect success rates, such as donor mare management, recipient mare quality and synchronization, and transfer technique/skills [7].

Success of ET is calculated based on two variables: embryo recovery rate and pregnancy rate after transfer of the embryo. Mean embryo recovery rate per cycle from single ovulating mares in commercial ET programs is approximately 50%, with higher rates for younger mares [5]. In addition, pregnancy rate after non-surgical transfer of embryos of excellent quality (Grade 1) range from 65 to 75%, resulting in a success rate for ET between 33 and 38% per cycle. Clinically, ET can be simplified by shipping the recovered embryo to a commercial ET center, avoiding concerns regarding availability and synchrony of recipient mares and transfer of the embryo. In addition, the process of cooling and shipping the embryos does not affect pregnancy rates compared to embryos transferred immediately after collection [8].

Common indications for ET include: (1) obtaining foals from performance mares, (2) obtaining multiple foals from individual mares each year, (3) obtaining foals from reproductively unsound mares, and (4) obtaining foals from mares with non-reproductive health problems that prevent them from carrying a pregnancy to term [3,9]. Most subfertile mares referred to an ET program are older mares with poor reproductive histories and unable to produce a foal by natural mating or AI [5]. For ET to be successful, the donor mare's reproductive tract needs to meet several requirements, as follows:

- Ovaries: grow a follicle and ovulate a healthy oocyte.
- Oviduct: transport gamete, support fertilization and transport embryo to the uterus.
- Uterus: provide an adequate environment to support embryo development until collection.
- Cervix: be patent and able to function properly during early pregnancy.

Mares with abnormalities that prevent conception or maintenance of the early embryo, such as unresponsive post-breeding endometritis, irreparable cervical lacerations, or uterine/oviductal scarring after dystocia, are not good candidates for ET [7]. In these cases, mares should be considered candidates for more advanced techniques such as OT, ICSI, or both.

3. Oocyte transfer (OT)

In the mare, oocyte transfer has been used to obtain pregnancies from valuable mares from which viable embryos cannot be obtained for transfer [10]. Removal of the oocytes from the preovulatory follicle before ovulation and transfer into healthy recipients is a good alternative in these cases. The first successful oocyte transfer in the horse was done in 1988 [11]. However, high success rates for oocyte transfer were not demonstrated until Carnevale and Ginther [12] obtained embryo development rates of 92% after transfer of oocytes from young donors into young recipients.

Collection of oocytes from ovarian follicles in the mare has been attempted by various methods, including laparotomy [13], flank transcutaneous punctures [14], and transvaginal, ultrasound-guided aspirations (TVA)[15]. Currently, the method of choice for follicular aspirations in our laboratory is TVA, using a linear ultrasound transducer [16]. In that regard, TVA has the advantage of being a non-surgical procedure, allowing repeated aspiration attempts without affecting subsequent fertility of the donor mare [17].

Clinically, oocytes are usually collected from the preovulatory follicle either at 24 or >30 h after induction of ovulation with gonadotropins [18]. Collection of immature oocytes is usually less successful, as the cumulus oocyte complex is tightly adhered to the follicular wall. Oocytes collected at 24 h require further incubation for 12–16 h to complete maturation. In contrast, oocytes collected >30 h after induction of ovulation can be immediately transferred into the recipient's oviduct [19].

The only requirement for the oocyte donor mare is to be able to grow a preovulatory follicle containing a healthy oocyte. The oocyte is then removed from the follicle before ovulation and transferred into a young recipient, where fertilization and embryo development will take place. Consequently, indications for OT include: ovulatory failure, failure of oocyte pickup, and severe pathology of the oviduct, uterus or cervix, including chronic uterine infections, cervical lacerations, or uterine/oviductal scarring.

Overall, OT is a more elaborate procedure than ET, requiring considerable investment in equipment and training. Because the oocyte is a single cell, it is more sensitive than embryos to changes in temperature, pH, osmolality, and physical damage. The OT procedure also requires cell culture equipment and good sterile technique to prepare media and manipulate oocytes. In addition, oocytes are transferred into the oviduct of recipients by flank laparotomy, requiring proper facilities for this procedure. Currently, only a few universities and large equine reproduction centers are successfully performing OT commercially. Similar to ET, success rate for OT depends on the oocyte collection rate and pregnancy rate per transferred oocyte. Oocyte collection rate from preovulatory follicles range from 65 to 75% and the mean pregnancy rate after transfer of oocytes from older donor mares is approximately 35% [20]. Therefore, success rates for OT range from 23 to 27% per cycle. Potentially, future development of non-surgical methods for transfer of oocytes, as well as better shipping protocols for transportation of oocytes, would help make OT a tool for practitioners to obtain pregnancies from subfertile mares in the field.

4. Intracytoplasmic sperm injection (ICSI)

Standard in vitro fertilization (IVF) has had only limited success in the horse, with only two foals being produced by this procedure in the early 1990s [21,22]. However, ICSI, a form of IVF, has provided a method to achieve fertilization in vitro in horses by injecting a single sperm into the oocyte [5,23].

Oocytes used for ICSI can be obtained ex vivo by aspirating the preovulatory follicle of a mare, similar to OT, or may be obtained by in vitro maturation of oocytes collected from small immature follicles, either ex vivo or post-mortem [23]. One of the main advantages of ICSI is that it only requires a very small number of sperm and, therefore, can be used to produce offspring from subfertile stallions with marginal-quality or low numbers of sperm. When using frozen semen for ICSI, a small section of one frozen straw can be thawed and used to produce multiple embryos, maximizing the use of valuable frozen semen [24].

Embryos fertilized by ICSI can be immediately transferred into the oviduct of recipient mares by flank laparotomy, or cultured for 7–8 d to reach the blastocyst stage. Blastocysts can then be transferred non-surgically into the uterus of a recipient mare. Alternatively, embryos can be cryopreserved and stored before transfer [25].

In addition to the complexity of the OT procedure, the ICSI procedure is extremely specialized, requiring expensive instrumentation and an experienced technician to perform the injections. To date, the clinical use of ICSI in horses has been limited by the cost of the procedure and the low success rates of oocyte collection and maturation. Oocyte collection rates for immature oocytes are usually <50%, but multiple small follicles can be aspirated per cycle [24]. In addition, only approximately 50% of these oocytes reach the metaphase II stage after in vitro maturation and can be subjected to ICSI [7]. Recently, Colleoni et al. [26] reported high oocyte collection (58%) and maturation (66%) rates, resulting in an average of 10 oocytes being collected per mare in their commercial ICSI program. One of the main advantages of ICSI compared to OT is that only oocytes that are capable to develop to blastocyst are transferred into the recipient mare. However, development to the blastocyst stage of injected oocytes is also low. In well-established centers, blastocyst development rates of 25–35% can be consistently achieved, with approximately 50% preg-

nancy rates after non-surgical transfer of embryos [27–29]. Future improvements in protocols for in vitro maturation of oocytes, as well as embryo culture, would allow ICSI to be more cost-effective, increasing its use in equine practice.

5. Summary

In conclusion, the decision of referring a mare to ART is a multifactorial task that depends on many variables, including the cause of subfertility, the value of the animal, and the owner's willingness to spend/invest considerable time and money attempting to obtain an offspring from their mare. The practitioner has an important role in educating the owner regarding risks and success rates of the technique to be used, in order to avoid client frustration and discredit of the ART. When advanced techniques are indicated to circumvent certain fertility problems, the mare should be referred to specialized centers in order to maximize the probability of success.

References

[1] Ginther OJ. Reproductive biology of the mare, 2nd edition, WI: Equiservices, Cross Plains; 1993.
[2] Oguri N, Tsutsumi Y. Non-surgical egg-transfer in mares. J Reprod Fertil 1974;41:313–20.
[3] Squires EL, Seidel GE Jr. Collection and transfer of equine embryos. Animal Reproduction and Biotechnology Laboratory Bulletin No. 08. Fort Collins, CO: Colorado State University, 1995.
[4] McCue PM, Squires EL, Bruemmer JE. Equine embryo transfer: techniques, trends and anecdotes. Proc Ann Conf Soc Theriogenol 2001;229–35.
[5] Squires EL, Carnevale EM, McCue PM, Bruemmer JE. Embryo technologies in the horse. Theriogenology 2003; 59:151–70.
[6] Vanderwall D. Current Equine Embryo Transfer Techniques. In: Ball BA, (Ed.), Recent Advances in Equine Reproduction. Ithaca: International Veterinary Information Service (http://www.ivis.org), 2000; Document no. A0204.0400.
[7] Hinrichs K, Choi YH. Assisted reproductive techniques in the horse. Clin Tech Equine Pract 2005;4:210–8.
[8] Carney NJ, Squires EL, Cook VM, Seidel Jr GE, Jasko DJ. Comparison of pregnancy rates from transfer of fresh versus cooled, transported equine embryos. Theriogenology 1991;36: 23–32.
[9] Aguilar J, Woods GL. Embryo transfer in horses: indications, technique, and expected outcomes. In: Youngquist RS, editor. Current therapy in large animal theriogenology. Philadelphia: WB Saunders Co.; 1997. p. 208–13.
[10] Carnevale EM, Alvarenga MA, Squires EL. Use of oocyte transfer in a commercial breeding program to obtain pregnancies from mares with reproductive pathologies. Proc 45th Ann Conv Am Assoc Equine Pract 1999; 200 (abstract).
[11] McKinnon AO, Carnevale EM, Squires EL, Voss JL, Seidel Jr GE. Heterogenous and xenogenous fertilization of in vivo matured equine oocytes. J Equine Vet Sci 1988;8:143–7.
[12] Carnevale EM, Ginther OJ. Defective oocytes as a cause of subfertility in old mares. Biol Reprod Mono 1995;1:209–14.
[13] Vogelsang MM, Kraemer DC, Bowen MJ, Potter GD. Recovery of equine follicular oocytes by surgical and non-surgical techniques. Theriogenology 1986;25:208.
[14] Palmer E, Duchamp G, Bezard J, Magistrini M, King A, Bousquet D, et al. Recovery of follicular fluid and oocytes of mares by non-surgical puncture of the preovulatory follicle. Theriogenology 1986;25:178.
[15] Cook NL, Squires EL, Ray BS, Cook VM, Jasko DJ. Transvaginal ultrasound-guided follicular aspiration of equine oocytes. J Equine Vet Sci 1993;15:71–4.
[16] Carnevale EM, Ginther OJ. Use of a linear ultrasonic transducer for the transvaginal aspiration and transfer of oocytes in the mare. J Equine Vet Sci 1993;13:331–3.
[17] Vanderwall DK, Hyde KJ, Woods GL. Effect of repeated transvaginal ultrasound-guided follicle aspiration on fertility in mares. J Am Vet Med Assoc 2006;228:248–50.
[18] Carnevale EM, Coutinho da Silva MA, Squires EL. How to collect and transfer oocytes. Proc Ann Conv Am Assoc Equine Pract 2003;49:293–4.
[19] Coutinho da Silva MA, Carnevale EM, Maclellan LJ, Seidel Jr GE, Squires EL. Effect of time of oocyte collection and site of insemination on oocyte transfer in mares. J Anim Sci 2002;80: 1275–9.
[20] Carnevale EM, Coutinho da Silva MA, Panzani D, Stokes JE, Squires EL. Factors affecting the success of oocyte transfer in a clinical program for subfertile mares. Theriogenology 2005;64: 519–27.
[21] Palmer E, Bezard J, Magistrini M, Duchamp G. In vitro fertilization in the horse. A retrospective study. J Reprod Fertil Suppl 1991;44:375–84.
[22] Bezard J. In vitro fertilization in the mare. Proc Int Sci Conf Biotech Horse Reprod 1992; 12 (abstract).
[23] Hinrichs K. Update on equine ICSI and cloning. Theriogenology 2005;64:535–41.
[24] Carnevale EM, Maclellan LJ. Collection evaluation and use of oocytes in equine assisted reproduction. Vet Clin North Am Equine Pract 2006;22:843–56.
[25] Campos-Chillon LF, Suh TK, Seidel Jr GE, Carnevale EM. Use of bovine embryos to establish methods for vitrification of early equine embryos. Theriogenology 2006;66:684–5.
[26] Colleoni S, Barbacini S, Necchi D, Duchi R, Lazzari G, Galli C. Application of ovum pic-up, intracytoplasmic sperm injection and embryo culture in equine practice. Proc Ann Conv Am Assoc Equine Pract 2007;53:554–9.
[27] Choi YH, Roasa LM, Love CC, Varner DD, Brinsko SP, Hinrichs K. Blastocyst formation rates in vivo and in vitro of in vitro-matured equine oocytes fertilized by intracytoplasmic sperm injection. Biol Reprod 2004;70:1231–8.
[28] Hinrichs K, Choi YH, Love LB, Varner DD, Love CC, Walckenaer BE. Chromatin configuration within the germinal vesicle of horse oocytes: changes post mortem and relationship to meiotic and developmental competence. Biol Reprod 2005;72: 1142–50.
[29] Hinrichs K, Choi YH, Love LB, Hartman DL, Varner DD. Transfer of in vitro-produced equine embryos. Proc Sixth Int Equine Embryo Transfer Symp 2005;38–9.