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Factors affecting the efficiency of foal production in a commercial oocyte transfer program

Fernando L. Riera [a], Jaime E. Roldán [a], José Gomez [a], Katrin Hinrichs [b,*]

[a] Laboratorio de Reproducción Equina Prof. Robert M. Kenney, Doña Pilar Embriones, Lincoln (B), Argentina
[b] Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, Texas, USA

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ABSTRACT

Transfer of donor oocytes to the oviducts of inseminated recipient mares (oocyte transfer, OT) presents a valuable method for production of foals from otherwise infertile mares. Little information is available, however, on factors affecting success of OT in a clinical setting. We report the findings over three breeding seasons in a commercial OT program developed at an equine embryo transfer center in Argentina. Overall, 25 mares were enrolled, and 197 follicle aspiration procedures were performed. The average mare age was 23 years. Follicle aspiration was performed with a needle placed through the flank; the oocyte recovery rate per follicle aspirated was 149 of 227 (66%). Induction of donor ovulation with deslorelin + hCG resulted in a significantly higher oocyte recovery rate than did induction with deslorelin alone (75% vs. 58%). There was no significant effect of mare age (17–20, 21–24, or 25–27 years) on oocyte recovery rate. Twelve oocytes were degenerating or lost during handling; transfer of the remaining 137 oocytes resulted in 42 pregnancies (31%) at 14 days. Of these, 32 (23% per transfer) went on to produce a foal or ongoing pregnancy. Transfer of oocytes recovered with a compact cumulus, without donor follicle induction, or less than 20 hours after induction was associated with a significantly reduced pregnancy rate (1/16, 6%), as was use of noncycling, hormone-treated recipients (2/22, 9%). To evaluate management factors affecting pregnancy rate, noncycling, hormone-treated recipients were disregarded, and only procedures using mature (expanded cumulus) oocytes recovered and transferred on the standard schedule (n = 99) were included. Mare age did not significantly affect rates of pregnancy or pregnancy loss. Similar pregnancy rates were obtained using recipients inseminated from 1 to 27 hours before transfer. Counterintuitively, insemination of recipients immediately (1–2 hours) after aspiration of the recipient follicle was associated with a high pregnancy rate (10/12, 83%). There was no significant effect on pregnancy rate of donor induction agent, the time the oocyte was in culture (2–20 hours) before transfer, time from recipient insemination to transfer, or total time from donor induction to transfer (32–45 hours). These findings establish that OT is robust, in that it is effective over a wide variation in timing of the different components involved, and can be successfully developed in a private embryo transfer practice.

1. Introduction

Embryo transfer (ET) is a widely used technique in the equine industry. Successful ET requires that the donor mare's tract must have the ability to transport semen, ovulate a viable mature oocyte, support fertilization within

* Corresponding author. Tel.: +1 979 862 1338; fax: +1 979 845 6544.
E-mail address: khinrichs@cvm.tamu.edu (K. Hinrichs).

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the oviduct, transport the resulting embryo to the uterus, and support the embryo in the uterus until the time it is recovered for transfer. Often, by the time a mare is recognized for its value as a broodmare, it may be older and have acquired infectious or traumatic damage to its tract that limits its ability to provide an embryo for ET. However, such mares may still have normal follicle development and viable oocytes. In these cases, oocytes may be obtained from the mares' follicles and fertilized *in vitro*. In the horse, because of the continued inefficiency of standard methods for IVF, this necessitates performance of intracytoplasmic sperm injection [1]. Unfortunately, the equipment and expertise required to perform equine intracytoplasmic sperm injection are extensive, and even when met, results can be disappointing [2].

Another technique for producing foals from isolated oocytes is oocyte transfer (OT), that is, the surgical transfer of the donor mare's oocyte to the oviduct of a recipient mare, and insemination of the recipient mare. This technique was developed in the horse because of the inability to effectively perform standard IVF. The procedures required for OT are feasible within the framework of a well-equipped equine veterinary practice. Experimental work with OT with oocytes from normal young mares and sperm from fertile stallions has shown that this technique can yield a high pregnancy rate (75%–83%) [3,4], and that the pregnancy rate can be equivalent to that for naturally ovulated oocytes in the same mares [5].

Despite the potential of OT as a clinical assisted reproduction technique, to the best of our knowledge, only two reports have been published on results of commercial application of OT, both from the same program. These reports presented results from 1998 to 1999 (64 transfers) [6] and 2000 to 2004 (504 transfers) [7]. The overall efficiency of the procedure in the two reports was 14% (14 foals from 99 donor mare cycles) and ~28% (159 pregnancies at 50 days from 570 cycles), respectively. Given that mares undergoing OT are those that have failed to provide an embryo for transfer and that mares in these programs were aged up to 30 years (mean of 19–22 years, and 19 years, for the two reports, respectively), the foals produced in these programs testify to the utility of OT for salvage of valuable genetics from known producing broodmares.

In the first of the aforementioned reports, efficacy tended to improve from the first to the second year (from 19% to 33% pregnancy per transferred oocyte), suggesting a learning curve for clinical application of the technique [6]. In the second report, the only factor found to significantly affect efficiency of commercial OT was time from administration of ovulation stimulant (deslorelin plus hCG) to transfer, with less than 32 hours providing poorer pregnancy rates than did longer periods. However, the authors discuss that this was complicated by the association of early transfer with recovery of oocytes from follicles with an ultrasonographic appearance suggesting impending ovulation or formation of a hemorrhagic follicle [7]. Other factors evaluated in that report were oocyte morphology, administration of oxytocin to the recipient mare, year, donor age, recipient type (noncyclic, hormone-treated [NCHT] vs. cyclic), and semen type and total motility; none of these parameters significantly affected results.

Performance of OT includes a number of component steps, including induction of donor mare follicle maturation, aspiration of the donor follicle, classification and culture of the recovered oocyte, aspiration of the recipient mare follicle, insemination of the recipient mare, and the actual surgical transfer of the oocyte to the recipient oviduct. The coordination of these components can be daunting to the inexperienced practitioner, and little information is available on the parameters within and among them that are associated with successful, or unsuccessful, OT. The purposes of the present article were to report the findings over three breeding seasons of a commercial OT program developed at an equine ET center in Argentina and to evaluate management factors affecting the efficacy of the procedure under these practice conditions.

2. Materials and methods

2.1. Donor mares

Commerical OT was conducted during the 2010 to 11, 2011 to 12, and 2012 to 13 breeding seasons (years 1, 2, and 3, respectively) in Buenos Aires province, Argentina. Follicle aspirations were performed from October to April of each year. Donor mares and stallions used were Polo Argentino type. Mare age is presented according to North American convention; e.g. if a mare turned 20 years old during a given breeding season, it was considered 20 years old for the entire season. Donors in the program for more than one season are recorded as the age they were during each analyzed season. The mares were housed at the Centro de Reproducción Equina Doña Pilar for the period they participated in the program. Donors were enrolled in the OT program due to repeated failure to provide embryos on uterine flush at this or other ET centers. After arrival, all prospective OT donors were examined by palpation and ultrasonography per rectum, and their history was evaluated. Mares carrying intrauterine fluid or having pyometra were treated with uterine lavage and/or administration of oxytocin; in refractory cases, a uterine swab sample was cultured for antibiotic sensitivity, and antibiotic treatment was initiated. If it was thought that there was a reasonable chance to produce an embryo by standard ET, this was attempted; otherwise, the mare was assigned to undergo OT.

Donor mares in diestrus (with a known ovulation date or having apparent luteal tissue visible on transrectal ultrasonography) were administered cloprostenol (Ciclase DL; Syntex S.A., Luis Guillón, Argentina) 390 μg, i.m., to induce luteolysis, or were allowed to come into estrus naturally. Ovarian activity was monitored by transrectal ultrasonography three to four times a week until a dominant follicle greater than 25 mm in diameter, accompanied by the presence of uterine edema indicating estrus, was detected. Thereafter, the ovaries were monitored ultrasonographically one to two times daily until ovulation-induction treatment and aspiration were performed. The ovulation-inducing agents, hCG (Ovusyn; Syntex), 1500 to 2500 IU, i.v. and/or deslorelin (deslorelin acetate; ChemPep, Inc., Wellington, FL, USA), 1.5 mg s.c., were administered to the mare when the follicle was judged to be likely to