

Embryo transfer in the dog and cat

R. Fayrer-Hosken*

Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, United States

Abstract

The practicality and feasibility of embryo transfer technology in dogs and cats is quickly becoming a clinical reality. Although progress has been slow, I anticipate that embryo transfer will be a practical and an economical technique in the near future. Most importantly, it is essential that the practical lessons learned with equine and bovine embryo transfer be integrated into the development of canine and feline programs.

© 2007 Elsevier Inc. All rights reserved.

Keywords: Embryo transfer; Superovulation; Oocyte; Dog; Cat

1. Introduction

The primary role of embryo transfer (ET) is to allow production of specific genetic offspring, especially in infertile females or those that cannot have their own offspring. In cattle and horses, genetic improvement through ET is an established and successful technology. However, ET in the dog is only successful experimentally [1,2]. In the cat, ET is a more straightforward and realistic technology, but costs preclude widespread application [3–6]. For additional information, consult a recent review [7].

Why has the development of ET in companion animals been much slower and less successful than in large domestic species? The major constraints, especially in the dog, are the control of endocrine timing and synchronization, embryo production and the technology of embryo transfer. Although research has slow and difficult, the need for viable clinical answers has lead to many practical solutions, including optimizing the timing of breeding, greater control of the female reproductive cycle and increased client demands to

perform more cutting-edge tasks. In the cat, there are far greater successes regarding control of the reproductive cycle, oocyte maturation, fertilization and transfer. Based on advances in equine and bovine ET technologies, it is expected that similar technologies will become more widely available for cats and dogs.

2. Endocrinology of the dog

The most important aspect of endocrinology, as related to embryo transfer, is optimal timing of embryo collection. The LH surge is triggered by a decline in the ratio of estrogen to progesterone. The pre-ovulatory LH surge lasts 24–40 h [8] (mean, 36 ± 5 h [9,10]) and causes an accelerated follicular development, partial luteinization of the granulosa-theca cell complex, and ultimately ovulation (that occurs approximately 30–36 h after the LH surge). Primary oocytes are ovulated; the first polar body is extruded 24–60 h after ovulation. Thus, the most fertile period of the bitch is from 3–5 days after blood progesterone concentration rises to >2.5 – 3.0 ng/mL. After the LH surge, there is a massive follicular commitment to progesterone production, resulting in rapid increases in blood progesterone concentrations.

* Tel.: +1 706 540 5473; fax: +1 706 742 7844.

E-mail address: rfh@vet.uga.edu.

The standard method for progesterone analysis is a radioimmunoassay (RIA). However, improvements in allied technologies have lead to increasing reliability in chemiluminiscent and enzyme-linked immunosorbent (ELISA) assays. It is noteworthy that an RIA should always be used as the gold-standard to validate these assays.

3. Timing breeding and embryo transfer in the dog

For embryo production and embryo transfer, timing of LH and ovulation are critical. In the dog, the typical timing protocol is to determine blood progesterone concentrations (using an RIA) beginning 7–8 days after the onset of proestrus, with samples collected every second day until there is definitive evidence of a competent LH surge. Based on an RIA, blood progesterone concentrations range from 0.5 to 2.0 ng/mL prior to the LH surge, with subsequent increases concurrent with increasing LH concentrations; as progesterone concentrations rise through 3 ng/mL, ovulation is beginning. After the LH surge, the rate of progesterone increase varies among individuals [10], in accordance with follicular development and the response to LH. Progesterone concentrations increase to the time of ovulation and fertilization; it is unlikely that across breeds there is specific progesterone concentration for breeding or embryo transfer. A progesterone plateau may be associated with ovulation, but on a practical basis, there is a sustained increase to peak progesterone concentrations (approximately 15–80 ng/mL), 15–25 days after the LH peak [8]. Intervals from the LH surge to parturition and from ovulation to parturition are 65 ± 1 days [11] and 63.9 ± 0.2 days [12,13].

4. Oocyte morphology, collection and maturation in the dog

Canine oocytes consist of dense cytoplasm bound by a plasma membrane. The cytoplasm contains the nucleus, or condensed chromosomes, and various organelles. During the interval that the oocyte is within the ovary, the Golgi apparatus plays an important role in yolk synthesis and formation of cortical granules; the latter are a key factor in preventing polyspermy [14]. The presence of marginated cortical granules along the plasma membrane is an indication of cytoplasmic maturity [15,16]. Spindles of microtubules form during meiosis and mitosis to aid in the alignment of chromosomes [16]. Cumulus cells are in close cellular contact (gap junctions) during maturation and provide nourishment to the oocyte. These cells also provide a surface for oviductal cilia during oocyte transport.

Oocytes are graded on a scale of 1–3, with Grade 1 darkly pigmented and completely surrounded by one or more layers of cumulus cells, Grade 2 lightly pigmented with incomplete layers of cumulus cells and Grade 3 pale, degenerate, frequently misshapen and devoid of cumulus cells. Stained oocytes were classified as germinal vesicle (GV, tight compact nucleus, Fig. 1), germinal vesicle break down (GVBD, nucleus less compact), metaphase I/anaphase I (MI/AI, presence of a metaphase plate/chromatids separating), metaphase II (MII, extrusion of a polar body, Fig. 2) or unidentifiable (U, unclassifiable nuclear material).

5. Superovulation, production and transfer of embryos in the dog

Yamada et al. reported obtaining embryos after IVF [17]; 54 bitches were treated with estrone, equine chorionic gonadotropin (eCG) and human chorionic

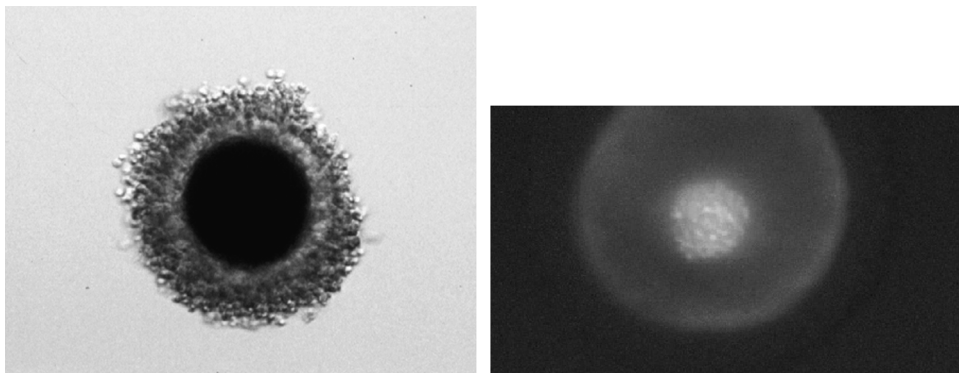


Fig. 1. Immature canine oocytes prior to in vitro maturation.

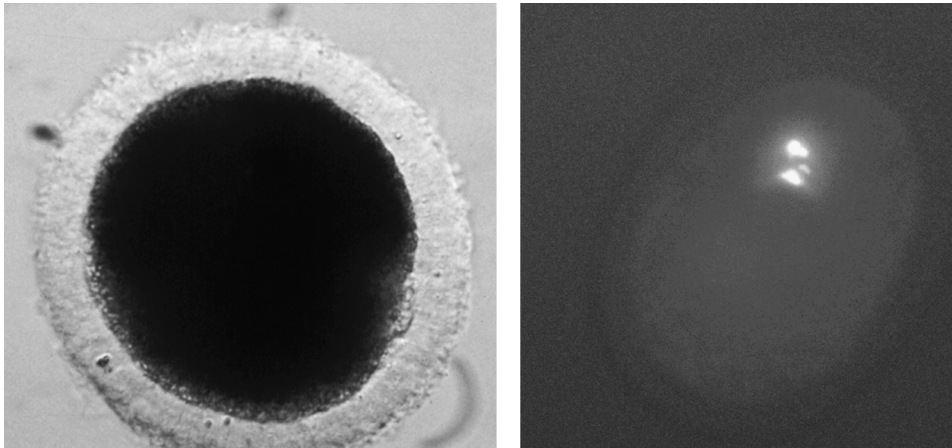


Fig. 2. In-vitro-matured canine oocyte at the metaphase II stage.

gonadotropin (hCG). Other stimulation techniques have utilized, FSH, estradiol and hCG. Embryos have also been produced to various stages through in vivo production [18–20], IVF [21] and ICSI [22]. For ICSI, in vitro maturation of oocytes 38.5% (82/213) reached the metaphase II (MII) stage. Oocytes were injected with sperm cells obtained from Percoll gradient-separated fresh semen. Of the ICSI oocytes, 7% (6/82) had evidence of pronucleus formation [22].

In dogs, embryo transfer is either surgical or non-surgical. Surgical transfer can be intra-tubal using glass catheters [18]; the oviduct is catheterized from the bursal/fimbrial end. Another surgical approach is intra-uterine placement of embryos, with an initial trochar puncture, followed by luminal catheterization. The most practical approach (for widespread clinical utilization), would be transcervical placement of appropriately staged embryos into a synchronized recipient.

6. Endocrinology of the cat

Although felids are photoperiodic and induced ovulators, gonadotropin stimulation using FSH or eCG and LH has proven successful. The most common protocol is to use daily doses (often decreasing) of FSH [6]. Superovulation of felids has been comprehensively reviewed [6,23,24] and has reduced the need for in vitro oocyte maturation. Oocyte maturation was very successful, even with superovulatory protocols [25–28].

7. Embryo production and transfer in the cat

Embryo production and transfer has been far more successful than in the dog, especially after the production of kittens [26]. Both ICSI and sub-zonal microinjection

of spermatozoa into the perivitelline space of cat oocytes have proven to be far more successful than the dog, with the production of several litters of kittens [5,6,29,30].

Standard embryo transfer with surgical intrauterine placement of embryos has been successful. The use of fresh or frozen embryos makes this technology realistic and practical. However, the primary impendence currently is the relatively small clientele for these services.

8. Conclusion

The creative and practical progress of embryo transfer technologies in the dog and cat is a balance of clinical need for these services and the relative need for the technology. Once there is more efficient oocyte maturation and fertilization in the dog, it is expected that the clinical availability of ET technology will expand substantially.

References

- [1] Kinney G, Pennycook J, Schriver M, Templeton J, Kraemer D. Surgical collection and transfer of canine embryos. *Biol Reprod* 1979;20:170.
- [2] Kraemer D, Kinney G, Schriver M. In: Embryo transfer in dogs and cats. Embryo transfer and in vitro fertilization. Second World Conference. 1989. p. 223–33.
- [3] Tsutsui T, Yamane I, Hattori I, Kurosawa N, Matsunaga H, Murao I, et al. Feline embryo transfer during the non-breeding season. *J Vet Med Sci* 2000;62:1169–75.
- [4] Pelican KM, Wildt DE, Pukazhenthi B, Howard J. Ovarian control for assisted reproduction in the domestic cat and wild felids. *Theriogenology* 2006;66:37–48.
- [5] Pope CE, Gomez MC, Dresser BL. In vitro production and transfer of cat embryos in the 21st century. *Theriogenology* 2006;66:59–71.

- [6] Pope CE, Gomez MC, Dresser BL. In vitro embryo production and embryo transfer in domestic and non-domestic cats. *Theriogenology* 2006;66:1518–24.
- [7] Luvoni GC, Chigioni S, Beccaglia M. Embryo production in dogs: from in vitro fertilization to cloning. *Reprod Domest Anim* 2006;41:286–90.
- [8] Concannon P, Hansel W, McEntee K. Changes in LH, progesterone and sexual behavior associated with preovulatory luteinization in the bitch. *Biol Reprod* 1977;17: 604–13.
- [9] Onclin K, Murphy B, Verstegen JP. Comparisons of estradiol LH and FSH patterns in pregnant and nonpregnant beagle bitches. *Theriogenology* 2002;57:1957–72.
- [10] de Gier J, Kooistra HS, Djajadiningrat-Laanen SC, Dieleman SJ, Okkens AC. Temporal relations between plasma concentrations of luteinizing hormone, follicle-stimulating hormone, estradiol-17 β , progesterone, prolactin and alpha-melanocyte-stimulating hormone during the follicular, ovulatory, and early luteal phase in the bitch. *Theriogenology* 2006;65: 1346–59.
- [11] Concannon PW, Hansel W, Vissek WJ. The ovarian cycle of the bitch: plasma estrogen LH and progesterone. *Biol Reprod* 1975;13:112–21.
- [12] Concannon P, Tsutsui T, Shille V. Embryo development, hormonal requirements and maternal responses during canine pregnancy. *J Reprod Fertil Suppl* 2001;57:169–79.
- [13] Concannon PW, McCann JP, Temple M. Biology and endocrinology of ovulation, pregnancy and parturition in the dog. *J Reprod Fertil Suppl* 1989;39:3–25.
- [14] Sato K. Polyspermy-preventing mechanisms in mouse eggs fertilized in vitro. *J Exp Zool* 1979;210:353–9.
- [15] Hyttel P, Callesen H, Greve T. Ultrastructural features of pre-ovulatory oocyte maturation in superovulated cattle. *J Reprod Fertil* 1986;76:645–56.
- [16] Barber MR, Lee SM, Steffens WL, Ard M, Fayrer-Hosken RA. Immunolocalization of zona pellucida antigens in the ovarian follicle of dogs, cats, horses and elephants. *Theriogenology* 2001;55:1705–17.
- [17] Yamada S, Shimazu Y, Kawaji H, Nakazawa M, Naito K, Toyoda Y. Maturation, fertilization and development of dog oocytes in vitro. *Biol Reprod* 1992;46:853–8.
- [18] Tsutsui T, Hori T, Kawakami E. Intratubal transplantation of early canine embryos. *J Reprod Fertil Suppl* 2001;57:309–14.
- [19] Tsutsui T, Hori T, Okazaki H, Tanaka A, Shiono M, Yokosuka M, et al. Transfer of canine embryos at various developmental stages recovered by hysterectomy or surgical uterine flushing. *J Vet Med Sci* 2001;63:401–5.
- [20] Tsutsui T, Hori T, Endo S, Hayama A, Kawakami E. Intrauterine transfer of early canine embryos. *Theriogenology* 2006;66: 1703–5.
- [21] England GC, Verstegen JP, Hewitt DA. Pregnancy following in vitro fertilisation of canine oocytes. *Vet Rec* 2001;148:20–2.
- [22] Fulton R, Durrant B, Keskintepe L, Fayrer-Hosken R. Assisted reproductive techniques for the treatment of canine infertility. Society for *Theriogenology* 1998;21.
- [23] Howard J. Assisted reproductive techniques in non-domestic carnivores. Philadelphia:Saunders 1999;449–57.
- [24] Pope CE. Embryo technology in conservation efforts for endangered felids. *Theriogenology* 2000;53:163–74.
- [25] Verstegen JP, Onclin K, Silva LD, Donnay I, Mettens P, Ectors F. Superovulation and embryo culture in vitro following treatment with ultra-pure follicle-stimulating hormone in cats. *J Reprod Fertil Suppl* 1993;47:209–18.
- [26] Goodrowe KL, Wall RJ, O'Brien SJ, Schmidt PM, Wildt DE. Developmental competence of domestic cat follicular oocytes after fertilization in vitro. *Biol Reprod* 1988;39:355–72.
- [27] Goodrowe KL, Hay M, King WA. Nuclear maturation of domestic cat ovarian oocytes in vitro. *Biol Reprod* 1991;45:466–70.
- [28] Kanda M, Oikawa H, Nakao H, Tsutsui T. Early embryonic development in vitro and embryo transfer in the cat. *J Vet Med Sci* 1995;57:641–6.
- [29] Pope CE, Johnson CA, McRae MA, Keller GL, Dresser BL. Development of embryos produced by intracytoplasmic sperm injection of cat oocytes. *Anim Reprod Sci* 1998;53:221–36.
- [30] Pope CE. In vitro fertilization and embryo transfer in felids. *Methods Mol Biol* 2004;254:227–44.