Embryo transfer in the dog and cat

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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.

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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.
The standard method for progesterone analysis is a radioimmunoassay (RIA). However, improvements in allied technologies have lead to increasing reliability in chemiluminescent 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 margined 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...
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 catherization. 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 impediment 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


