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In vitro embryo production and embryo transfer in domestic and non-domestic cats

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Abstract

Over a 5-year interval, multiple laparoscopic oocyte retrievals were done in fishing cats (*Prionailurus viverrinus*), caracals (*Caracal caracal*) and domestic cats after ovarian stimulation with gonadotropins. From 21 retrievals in five fishing cats, 579 preovulatory oocytes (mean = 27.6) were recovered and 348 embryos were produced in vitro (mean = 16.6). A total of 452 preovulatory oocytes (mean = 25.1) were recovered from 18 of 24 retrievals in six caracals and 297 (mean = 16.6) embryos were produced. An additional 16 caracal embryos (19%) were produced after in vitro maturation of 83 oocytes, 59 of which came from six retrievals producing only immature oocytes. The presence of corpora lutea at oocyte retrieval occurred in each species (1) at a similar frequency (33%) and (2) more frequently during January through May (11 of 15 retrievals) than during the latter half of the year (4 of 30 retrievals). Of the 12 embryo transfer procedures done in fishing cats, one pregnancy (8%) was obtained and one live kitten born after the auto-transfer of 10 Day-6 embryos. In caracals, a total of 46 Day-4 or Day-5 embryos were auto-transferred to six recipients, one of which delivered two live kittens. Then, 109 caracal embryos were cryopreserved before thawing and transferring to nine recipients (mean = 12.1) on Days 5 or 6. From three pregnancies established (33%), a total of three kittens were born. Two to six gonadotropin treatments/oocyte retrievals were done in domestic cats during 1999 through 2003; an average of 24.9, 23.5, 22.0, 23.1, 23.5 and 40.9 oocytes ($P > 0.05$) were recovered at the first through the sixth treatment cycles from 138, 138, 97, 49, 22, and seven retrievals, respectively.

Keywords: Cats; IVF; Embryo transfer; Cryopreservation; Oocyte retrieval

1. Introduction

There was an approximately 10-year interval between the first report of the birth of kittens after embryo transfer (ET) in the cat [1] until the birth of kittens after IVF/ET [2] and after embryo cryopreservation/ET [3]. Since then, techniques for the in vitro production of cat embryos have developed sufficiently to allow up to one-half of all in vitro embryos to develop into blastocysts in vitro [4–6] and births of kittens after transfer of embryos derived by a variety of in vitro techniques [7,8], including somatic cell nuclear transfer [9,10]. This increase in activity and the subsequent progress may be attributable to several factors, not the least of which is a greater awareness that many felid species are at risk of extinction. The recent advancements have allowed exploration of the possibility of using assisted reproductive technology for supporting perpetuation of genetically valuable non-domestic felids. Therefore, the primary purpose of the

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present study was to evaluate the response of two species of non-domestic cats – fishing cats and caracals – to multiple gonadotropin hormone treatments/laparoscopic oocyte retrievals by determining the (1) number of preovulatory oocytes produced per retrieval; (2) frequency of cleavage after in vitro fertilization and (3) establishment of pregnancy/birth of kittens after embryo transfer. For comparative purposes, we are also presenting the results on preovulatory oocyte production following multiple gonadotropin hormone stimulations/laparoscopic oocyte retrievals in our colony of domestic cats over a 5-year interval.

2. Materials and methods

2.1. Animals

Domestic cats were antibody-defined animals purchased from a USDA approved, AAALAC accredited vender (Liberty Research, Waverly, NY, USA). They were group-housed in rooms maintained at 72–76 °F and a 14/10 h cycle of light/dark. The rooms were cleaned and fresh food and water were provided daily. The first group of 40 multiparous females (5–6-year-old) arrived in late 1998. Subsequently, cats were added to the colony in groups of 20–30 nulliparous females that were <1 to 3 years of age at arrival.

Six caracal and five fishing cat females were used during the period from April 1999 to March 2004. They were housed individually in large out-door enclosures and fresh food was provided daily with water always available. All animal procedures were approved by the Institutional Animal Care and Use Committee of the Audubon Nature Institute as required by the Health Research Extension Act of 1985 (Public Law 99-158).

2.2. Semen collection and storage

Caracal and fishing cat semen was collected using a standard electroejaculation technique [11]. An aliquot of each fresh semen sample was extended separately in TEST yolk buffer (TYB; Refrigeration Medium, Irving Scientific, Santa Ana, CA, USA), cooled slowly to 4 °C, and held overnight to be used for IVF the following day. The remainder of the sample was extended in TYB, cooled to 4 °C, extended (1:1) with TYB containing 12% glycerol (Freeze Medium, Irving Scientific) using a modified fixed molarity multi-step method [12], loaded into 0.25 mL straws, and frozen on dry ice for 10 min before storage in liquid nitrogen. Straws were warmed in a 65 °C water bath for 5 s and Hepes buffered (15 mM) Tyrode's solution (HeTy) containing pentoxifylline was added to the sample incrementally in seven steps, using a modified fixed-molarity method [12]. The diluted sample was centrifuged at 200 × *g* for 10 min, the supernatant removed and the sperm pellet was resuspended in HeTy.

2.3. Ovarian stimulation with exogenous gonadotropins

Lyophilized porcine FSH and LH (Sioux Biochemical, Sioux Center, IA, USA) was reconstituted with a sterile solution of 2% carboxymethyl cellulose and 1% Tween 20 in water and either stored at 4 °C for use within 2 days or stored at −80 °C for later use. Females were treated with gonadotropins and oocyte retrievals were done from one to eight times, with at least a 6-month interval between successive gonadotropin treatments/oocyte retrievals. Domestic cat oocyte donors were interestrus females treated with a total of 3–5 IU of FSH administered SQ in daily decreasing doses for 4 days, followed on the fifth day with 3 IU LH (IM). Six caracal females (average weights = 9.8–13.4 kg) received a total of from 15 to 25 IU of FSH and five fishing cat females (average weights = 9.2–11.5 kg) received a total of 7.5 IU FSH given as decreasing daily treatments over 4 days. On Day-5, caracals and fishing cats were administered 10 or 15 and 5.0 or 7.5 IU of LH, respectively. From April 1999 to March 2004, 21 and 21 laparoscopic oocyte retrievals were done on the five fishing cats (three to six per female) and three caracals (six to eight per female), respectively. In 2003, three procedures were done on three additional caracal females, for a total of 24 caracal oocyte retrievals on six females.

2.4. Oocyte recovery

Oocytes were recovered from all donor females by laparoscopy at 24–25 h after LH administration. The laparoscopic procedure has been described in detail [8]. Follicle contents were aspirated into a 15 mL tube with TL Hepes medium containing 10 IU/mL heparin. Oocytes surrounded by an expanded cumulus cell mass (preovulatory) were rinsed and pooled in Hepes buffered modified TCM 199 + BSA (H-199) at 38 °C until IVF. Morphologically intact caracal oocytes, partially or fully surrounded by compact corona radiata cells, were placed into IVM medium for 24 h before doing IVF.