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In vitro production and transfer of cat embryos in the 21st century

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Abstract

Appreciable progress has been made in the development of assisted reproductive technology (ART) for creating in vitro embryos in cats. Moreover, the extent of advancement in the last decade has been similar, albeit of more modest magnitude, to that seen in some other domestic and laboratory species, particularly when the disparities in financial, and, hence, scientific, resources are considered. The recent progress in domestic felid ART has made it possible to envisage their potential role in supporting the conservation of endangered felid species, which, in reality, is a multifarious process requiring wide-ranging, yet coordinated approaches. The prospect of incorporating ART into that intricate domain, with limited exceptions, remains a long-term, but highly motivating objective. Meanwhile, the straightforward accessibility and abundant supply of domestic cat gametes from local veterinary clinics provides a valuable and practical source of material for further research on the basic aspects of in vitro oocyte maturation, fertilization and early embryo development. Furthermore, extrapolating the domestic biotechniques to non-domestic felids has produced encouraging results in some species.

Keywords: Cats; In vitro maturation; In vitro fertilization; Embryo culture; Embryo transfer

1. Introduction

International interest in cat ART is expanding, due perhaps, in part, to the recent births of viable kittens following transfer of embryos derived by somatic cell nuclear transfer (SCNT; [1–3]), a subject addressed by another contributor to this special issue. Several excellent reviews on ART in carnivores have been published in the last few years [4,5], some of which particularly focused on their application to non-domestic species [6–8]. The most recent reviews specifically dedicated to assisted reproduction in cats were published in 2000 [9], 2001 [10] and 2003 [11]. Therefore, the purpose of the present report is to summarize the more recent literature on cat in vitro oocyte maturation, in vitro fertilization, in vitro embryo culture and associated techniques, such as cryopreservation and intracytoplasmic sperm injection, an array of integrated procedures known collectively as in vitro production of embryos. Research results published before 2000 will be mentioned only to provide relevant background information. In addition, a summary of our results and other recent literature on response of domestic and some non-domestic cats to repeated gonadotropin stimulation of follicular development/oocyte retrieval will be presented. Finally, recent studies on the ability of in vitro derived embryos (excluding SCNT derived embryos) to establish pregnancy and result in births of kittens after transfer to recipients will be described, including results produced by applying the technology to non-domestic cats.

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2. In vitro oocyte maturation and embryo culture

2.1. Source of oocytes

2.1.1. In vivo versus in vitro maturation of oocytes

The range of oocyte/embryo developmental events that can be successfully achieved in vitro continues to expand. However, the probability that the resultant embryo will produce a viable fetus diminishes in proportion to the extent of developmental events that were involved in producing it occurred in vitro. For example, it is becoming increasingly clear that the environment of the cumulus oocyte complex during meiotic maturation from the germinal vesicle stage (prophase I) to extrusion of the first polar body (metaphase II) plays a fundamental role in subsequent development of the embryo. A good illustration of this phenomenon is the reduction in cleavage frequency and blastocyst development of cat embryos produced from in vitro matured oocytes, as compared to that of embryos derived from in vivo matured oocytes recovered from preovulatory follicles [12]. The dissimilar in vitro developmental competence has been attributed to a lack of cytoplasmic maturation in oocytes matured in vitro [12]. Bogliolo et al. [13] demonstrated that M-phase promoting factor (MPF) and mitogen activated protein kinase (MAPK) activities were lower after in vitro maturation than after in vivo maturation of cat oocytes. Both MPF and MAPK are implicated in the process of oocyte maturation, and their level of activity controls meiotic progression from germinal vesicle breakdown to arrest at metaphase II. Peak levels of MPF and MAPK occur at approximately the same time as germinal vesicle breakdown (12 and 8 h, respectively). Furthermore, duration of in vitro maturation influences levels of MPF and MAPK kinases, being higher at 24 h than at 40 h of culture.

2.1.2. Growth factor supplementation of IVM medium

The beneficial effects of supplementing oocyte maturation and embryo culture medium with growth factors, particularly epidermal growth factor (EGF) and insulin-like growth factor (IGF-1), have been widely demonstrated in several species; however, there are few reports on their use in cats. Although the addition of 10 ng/ml of EGF to IVM medium did not significantly improve cleavage frequency after IVF of high quality oocytes (53% versus 48%), the percentage of embryos that developed into blastocysts was increased (55% versus 43%; $P < 0.05$) [14]. The effects of various concentrations (0, 10, 25 or 50 ng/ml) of EGF during IVM on in vitro developmental competence of cat oocytes were evaluated by Merlo et al. [15]. They found that cleavage frequency and blastocyst development were higher ($P < 0.01$) in the 25 ng/ml EGF group than in the control (0 ng/ml) and 50 ng/ml EGF groups and concluded that adding EGF to the maturation medium enhanced cytoplasmic maturation.

In perhaps the only report examining the effects of adding IGF-I to cat oocyte maturation medium, Kitiyanant et al. [16] found that the percentage of good quality oocytes completing nuclear maturation to telophase I and metaphase II was increased by supplementation with 100 ng/ml IGF-I (52–66% versus 70–86%). The beneficial effect was not apparent in lower quality oocytes with partial or no cumulus cell layers. The good quality oocytes undergoing IVM in IGF-I supplemented medium were subsequently used successfully in nuclear transfer experiments in which 3–8% developed to blastocysts, depending on the type of donor somatic cell used.

2.2. Culture environment

The recent surge of interest in producing cat embryos in vitro is represented by both an increase in quantity of publications and in the number of laboratories from which the research is originating. Earlier, the dissimilar approaches taken by the few laboratories active in this area produced some obvious dichotomies in the results obtained. More recently, the combination of enhancements in previously established in vitro methods, including adaptation of protocols widely used in other species, are generating consistent improvements in the in vitro production of cat embryos [17–19]. While acknowledging that cat embryos do possess their own pattern of eccentricities, in spite of some suggestions to the contrary, the complexities encountered in producing cat embryos in vitro are fundamentally similar to those experienced when dealing with preimplantation in vitro embryogenesis in other mammalian species. For instance, considerable concern has been shown for an in vitro developmental block occurring in cat embryos at the morula to blastocyst transition [20] that was resistant to modifications in the culture environment [21,22]. Yet, in recent studies from other laboratories, the proportion of embryos developing to the blastocyst stage often approaches that reported in more widely studied species, ranging from 40% to ≥60% of embryos [17,18,23]. Furthermore, these results were obtained by culturing cat embryos in three different media: synthetic oviduct fluid (SOF) supplemented with amino acids and