INTRODUCTION

If you received a copy of the proceedings from last year’s SFT meeting, you will notice the title for this presentation is the same. Due to the tragedy of September 11th, the SFT meetings were cancelled, and in turn my presentation on this topic. Since that time there have been no reports of a dog being cloned, thus the title remains appropriate as the question posed is still of great interest to many.

The goal here is to provide an abbreviated update of progress that has occurred over the past year as pertains to animal cloning in general, and where possible specifics as relate to research involving dogs. Current methods for cloning animals involve nuclear transplantation. In its simplest form, nuclear transplantation comprises transferring a nucleus from one cell to another. For cloning animals this entails transferring the nucleus of a cell obtained from the individual to be cloned into an unfertilized ovum that has had its metaphase chromosomes removed. Once this occurs, the nucleus is reprogrammed, and utilized by the egg cytoplasm to direct development of a new embryo. This embryo is genetically identical to the animal from which the original donor cell was obtained and can be transferred into a surrogate female for gestation to term and birth of a clone (Campbell et al, 1996; Wilmut et al, 1997; Cibelli et al, 1998; Kato et al, 1998; Wells et al, 1998; Wakayama et al, 1998, Wakayama and Yanagimachi, 1999; Kubota et al, 2000; Hill et al, 2000a; Baguisi et al, 1999; Keefer et al, 2000; Polejaeva et al, 2000; Onishi et al, 2000).

CLONING DOGS BY NUCLEAR TRANSFER

It has now been more than three years since we initiated our research focused on cloning dogs by nuclear transplantation. We have not yet produced a cloned dog; neither have there been any reports from other laboratory groups claiming success. Progress has been slow yet steady. One major milestone was achieved this past year with the production of our first pregnancy following the transfer of a cloned dog embryo. The pregnancy was detected on day 26 of gestation, appeared completely normal and the fetus exhibited a strong heartbeat. On day 38 of gestation for reasons unknown, the heart ceased to beat; therefore a decision was made to surgically remove the fetus. The conceptus and extraembryonic tissues were processed for analysis to try and determine the cause of death. A canine fetus measuring about 6.5 cm in length was recovered from the uterus. Tissues were snap frozen for DNA analysis, which subsequently confirmed the fetus was a clone. To date, we have not detected anything abnormal in the tissues that were recovered and have been unable to determine the cause of death.
Other than producing this single cloned pregnancy, little has changed this past year that could be labeled as a “major breakthrough”. Progress towards developing technology for cloning dogs by nuclear transfer is still hampered by two main obstacles 1) obtaining mature dog ova that can be used for nuclear transfer to produce cloned embryos and 2) estrus induction so to prepare embryo recipients (surrogate bitches). In order to provide some appreciation for this challenge, we maintain approximately 70 dogs in our colony that are available for use in our research program. From February 2001 through December 2001 only 40 dogs exhibited estrus (either natural or induced) and were utilized to collect ovulated mature ova for nuclear transfer and/or as embryo recipients. In total, 150 ova were recovered (average 3.85 per collection), but only 73 (average 1.8 per collection) were judged to be viable. These ova were used for nuclear transfer resulting in 64 embryos that were transferred into 22 recipient females along with 57 additional cloned embryos produced using ova obtained by in vitro maturation. In summary, over a period of 12 months, only 121 cloned embryos were produced and transferred into 22 recipients resulting in a single conceptus that survived through 38 days of gestation.

It is reasonable to assume, based on our progress to date, and data from research involving other species, that chances of producing a cloned dog are highly correlated with the number of cloned embryos that can be produced and subsequently transferred into recipient females. Cloning animals by nuclear transplantation remains inefficient in all species; often times requiring the transfer of hundreds of embryos into recipient females to obtain a single offspring. Producing and transferring hundreds of cloned dog embryos into surrogate bitches continues to represent an extremely difficult challenge. As I indicated in last year’s proceedings, the primary problem is not with the nuclear transfer procedure itself, rather the inability to obtain large numbers of mature canine ova. Given that a bitch cycles only once every 6 – 12 months, and protocols for inducing ovulation have proven unreliable and inconsistent, collection of in vivo matured (ovulated) ova to use for nuclear transfer is unlikely to result in an adequate number of ova unless a large number of females (hundreds) are available for use. The most effective and economical solution to this problem is in vitro oocyte maturation using canine ova obtained from ovaries of bitches that are spayed. Unfortunately, little progress has been made towards developing effective methods for producing viable canine ova by in vitro maturation. This past year we utilized literally thousands of immature ova collected from canine ovaries to test a variety of different methods for obtaining in vitro maturation. Modifications to maturation medium including the addition of growth factors, hormones, and other compounds previously used in other species to increase maturation rates, proved ineffective, as did increasing the amount of time in culture. Regardless of the system used for in vitro maturation, the proportion of canine ova maturing to metaphase II approximated only 5%-10%.

In addition to our studies involving in vitro oocyte maturation and nuclear transfer, we have also continued to explore different techniques for inducing ovulation in dogs. As with oocyte maturation, progress in this area has also been limited. Different compounds that have been used include Lupron, HMG, GNRH, and Cabergoline. None of the protocols we tested have proven satisfactory. Most of our efforts have involved the use of Lupron, a GNRH agonist that was previously reported to result in fertile estrus in later anestrous bitches (Inaba et al, 1998). Of 60 bitches used in one study, only 36 exhibited estrus and of these only 26 ovulated. In total, 121 ova were collected (average 2 ova per bitch receiving treatment).
CLONING DOGS NOW AND IN THE FUTURE

As was the case a year ago, the answer to the question “when will the first dog be cloned?”, remains unknown. Likewise, a reasonable prediction is still somewhere between 60 days and 6 years. Obtaining a pregnancy from a cloned dog embryo is encouraging, however, this does not represent a normal healthy animal, and it is common knowledge that many pregnancies resulting from cloned embryos do not survive to term.

Some of the most pertinent information pertaining to the future of cloning dogs can be derived from research conducted this past year involving other species. Two new animal species, rabbits and a cat, were cloned using adult cells (Shin et al, 2002; Chesne et al, 2002). The first cloned cat was produced in our laboratory and is perhaps the most relevant as cats are carnivores; also gestation length and placentation are similar to dogs. In last years proceedings I predicted that a cloned cat would be produced before the first cloned dog. This is because a number of different laboratory groups are conducting research focused on cloning cats. More significant, many of the assisted reproductive technologies including in vitro oocyte maturation, in vitro fertilization, in vitro embryo culture, embryo transfer, and induction of estrus have already been developed and proven effective in cats. In contrast to dogs, it is relatively easy to produce large numbers of cloned cat embryos by using in vitro matured ova for nuclear transfer and then transfer these embryos into recipient queens in which ovulation has been induced by hormone injections.

Cats and dogs are also similar in that they both represent pets. In light of the birth of the first cloned cat, pet cloning has become even more controversial. Many animal welfare groups such as the Humane Society of the United States are strongly opposed to cloning pets. However, many individual pet owners or others interested in cloning cats and dogs for biomedical research purposes are supportive. It is interesting to note that much of the opposition to cloning pets has been founded on the notion that it will contribute to the problem of pet overpopulation. This is highly unlikely, and in contrast, there is a much greater probability that research in this area will eventually contribute to solving the problem of pet overpopulation. In dogs research focused on cloning requires scientific investigation of reproductive physiology in canids. Much of the basic knowledge gained from these studies can be used to develop new methods for pet contraception. For example, understanding the requirements for normal oocyte growth and development in turn provides information that can be used to try and inhibit or interfere with this process. Knowledge gained by ovulation induction studies may result in new methods to prevent ovulation and/or estrus from occurring. These represent only two of many potential targets.

There also seems to be some misconception of the scale at which pet cloning might someday be applied. This is perhaps brought on by the word itself, “clone”, which for some reason puts to mind the production of thousands of genetically identical individuals. First, the cost itself will greatly limit the number of people that might want to clone their pet as it will never be cheap, and even if the process becomes much more efficient, will likely cost thousands of dollars. Also, if an individual can afford it and decides to clone their pet, they will probably want only one animal. More significant in terms of the problem with pet overpopulation is the need for mature oocytes to produce cloned embryos. As mentioned above, the only reasonable approach for obtaining large numbers of ova to use for nuclear transfer is to utilize ova obtained from animals.
that are spayed, and mature these in vitro. Even if cloning pets becomes a relatively efficient process, a large number of animals will need to be spayed to provide the necessary ova to produce a single clone. These ova will not come without cost, rather, cooperative agreements will be formed between institutions needing the ova for cloning and those providing spay services, with funds flowing to spay and neuter clinics to help offset costs. Alternatively, institutions needing ova for cloning will establish their own discount spay and neuter clinics so to obtain ova. In our case for example, the private individual supporting our research has already provided hundreds of thousands of dollars to support the clinic from which we obtain ova for our research. Again, it is much more likely that cloning cats and dogs, whether for research purposes or pet cloning, will do more to help solve the problem of pet overpopulation than it will ever do to contribute to it.

Another criticism of cloning cats and dogs (pets) is founded on the knowledge that some cloned animals die at birth or shortly thereafter due to developmental abnormalities (Hill et al, 1999; Hill et al, 2000b). In addition, a number of reports involving research with mice have now demonstrated more long-term effects including problems with obesity and hepatic disease leading to early death (Tamashiro et al, 2002; Ogonuki et al, 2002). These are legitimate concerns and certainly must be addressed with additional research prior to the commercial application of pet cloning. The reason some cloned animals exhibit developmental abnormalities is at present unknown, however recent information points to inadequate reprogramming of nuclei during the process of nuclear transfer leading to abnormal expression of genes (Kook Kang et al, 2001; Bourc'his et al, 2001; Dean et al, 2001). While some clones exhibit developmental abnormalities, others appear normal (Baguisi et al, 1999, Keefer et al, 2000; Lanza et al, 2001). This suggests that perhaps differences between species or the methods used for nuclear transfer are affecting the outcome. At the same time, it also suggests that with enough time and research, the problems and inefficiencies currently seen in animal cloning will be overcome and cloning animals will be rather routine.

On a final note, the applications for cloning dogs include not only the possibility of cloning pets but also the genetic replication of superior service animals such as seeing-eye dogs and/or those used for search and rescue. Other applications include conservation of endangered species and the creation of models for both animal and human disease. As I mentioned in the beginning, the SFT meetings were cancelled last year due to the tragedy of September 11th. Ironically, it was this same day that we confirmed our first cloned dog pregnancy. Even after working for 3 years and spending millions of dollars to reach this goal, as I sat at home watching the events on TV unfold, a phone call letting me know that we had finally produced a pregnancy just didn’t seem that important or significant. Having said that, knowing that a large number of search and rescue dogs were used in recovery efforts following 9/11/01, and many more serve in the military or various law enforcement agencies, one has to wonder what role cloned dogs might play in our future.

REFERENCES


