ORAL COMMUNICATIONS

Animal Biotechnology

[A.1]

Biotechnological opportunities in veterinary interventions and challenges in their Global adoption: the research perspective

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During the last 40 years studies in the mouse, including the production of chimeras, development of pronuclear injection techniques and the establishment of embryonic stem (ES) cells have provided models for the study of development and differentiation, precise and routes for genetic modification of the nuclear genome. The use of ES cells combined with homologous recombination allowed precise genetic modifications of the mouse genome including gene knockouts, knockins and modification of specific sequences producing models to study function of individual genes and their role in disease. Over this period parallel studies in other species were less successful, pronuclear injection produced transgenic animals in a range of species, however, this technique is limited to gene addition. The isolation of ES cells in domestic species would provide a more precise route to genetic modification although the extended generation times of many species as compared to the mouse may limit its use. To date, attempts to isolate ES cells have only proved successful in humans and monkeys. The development of somatic cell nuclear transfer provided novel opportunities not only for animal production but also for precise genetic manipulation. Continued research on adult derived stem cells, epiblast stem cells and studies on reprogramming which lead to the production of induced pluripotent cells (iPS). All of these technologies provide routes to the modification of domestic species in a range of basic and applied research including; models for human disease and cell based therapeutic medicine, biopharmaceuticals, modification of animal traits, genetic intervention for disease prevention and cell based therapies for animal medicine. However, application of these technologies is slow, in part this may be due to cost, but, in addition to economic considerations social, ethical and religious factors need to be discussed and addressed. This paper will review the potential biological opportunities provided by these technologies in domestic species and discuss factors restricting their global acceptance.

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[A.2]

Cloning of anatolian grey cows

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Keywords: Nuclear transfer; Cloning; Bovine embryo

In the present study, cloning of native Anatolian Grey Cows living semi-wildly especially in Marmara Region was aimed. Cartilage, fibroblast and granulosa cells obtained from the ear tissue and ovarian follicles of an Anatolian Grey Cows as nuclear material source and oocytes isolated from slaughterhouse ovaries from Holstein cows as cytoplasm source were used. NT units were cultured in Sage® medium supplemented with BSA and FCS for 7 days. Development rate to blastocyst of embryos from granulosa cells (33.33%; 90/270) was significantly higher than the rate of embryos from cartilage cells and fibroblast cells (21.5%; 134/623, 19.48%; 30/154 respectively). Thirty two embryos from cartilage cells, 10 embryos from fibroblast cells and 15 embryos from granulosa cells were transferred into recipient cows (1–2 blastocysts/a recipient cow). Day 35 pregnancies were diagnosed in 10 cows from cartilage cells (43.48% 10/23), in two cows from fibroblast cells (33.33% 2/6) and in four cows from granulosa cell (36.36% 4/11). One healty male calf from fibroblast and two female calves from granulosa cells were born healthy and normal weight. Genotyping by using 11 microsatellite DNA loci was shown that the calves had the same genotype with the donor cells. In addition, partial mtDNA PCR sequencing results had shown that the calves and the donor cells did not have the same mtDNA type. Furthermore, the hetoroplasmy analysis based on SSCP and RFLP methods revealed no mtDNA heteroplasmy. Finally, telomer length analysis did not reveal significant results indicating short telomer lengths in the cloned calves. VEGF and IGF-1 stainings were observed in epitelial region of clon and control plasentas and the leptin antibody was not found in clon and control groups as the pregnancies lasted more than 150 days by immunohistochemistry analyzes.

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[A.3]

Correlations between Growth Traits and Heterozygosity, Allelic Distance (d^2) at Microsatellite Loci in the Yellow Perch, *Perca flavescens*

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Keywords: Heterozygosity-fitness correlations (HFCs); Growth traits; Microsatellites; Yellow perch

The correlations between individual genetic heterozygosity observed at marker loci and fitness-related traits (HFCs) have been studied in a number of taxa. Although significantly positive HFCs have been observed in many organisms, they are not universal. Their strength and stability vary according to species, populations, ages and sexes. Yellow perch, *Perca flavescens*, is an important aquacultural and recreational fish species in the United States and Canada. Because of the high market demand and dramatic reductions in population sizes of yellow perch, this species holds tremendous potential for aquaculture in the USA. Advanced knowledge of HFCs and its relation to heterozygosity are important for species conservation and selective breeding (heterosis). The objective of this study was to determine the relationships between individual genetic heterozygosity and growth traits in cultured yellow perch.

1165 individuals were genotyped with eight microsatellite markers. Using regression analyses, correlations between genetic parameters (microsatellite heterozygosity and mean square allelic distance (d^2)) and total length, body weight of yellow perch reared

in different culture conditions (four ponds), ages (one-year-old and two-year-old), breeding methods (one-stage selection and two-stage selection) and sexes were assessed.

There were no significant associations found between heterozygosity, d^2 and growth traits of yellow perch reared in different culture conditions, ages, breeding methods and sexes (except one, where *P* value for correlation coefficients was 0.046; for all the others, *P* values were greater than 0.05, ranged from 0.063 to 0.975).

These results suggested that i) the general thoughts of heterozygosity and heterosis might be rejected in yellow perch, or ii) the microsatellites used here might be located in genes or in the proximity of genes uncoupled with growth. Moreover, another hypothesis also could be proposed that the heterozygote advantage might be found in yellow perch fingerlings since the HFCs are expected to decrease or disappear with age.

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[A.4]

Molecular diversity in the nucleocapsid protein of feline coronaviruses (FCoVs)

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Keywords: Feline Infectious Peritonitis; Feline coronaviruses; N protein; Phylogenetic analysis

Introduction: Feline coronavirus (FCoV) infections are extremely common in cats worldwide. Most natural infections are subclinical and result in self-limiting gastrointestinal disease (cases infected with feline enteric coronavirus FECV). Only a small percentage of infected cats develop the classical symptoms of feline infectious peritonitis (FIP), a fatal immune-mediated disease which is caused by a virulent variant of FCoVs, the FIP virus (FIPV biotype). Specific genetic determinants of these clinical outcomes have yet to be discovered. FIPV was distinct from FECV in disease potential but both viruses co-existed in the same population and were antigenically identical.

Several studies suggested a role of the N protein in stimulating a cell-mediated immunity against FCoV, the only immunity which appears to play a protective role. In the light of the putative immunogenic role of the N protein, the major goal of this study was to investigate the molecular diversity of the N protein and to give evidence sites ("hot spots") subject to selective pressure in FCoV strains detected in healthy and diseased cats.

Methods: The nucleotide sequence of N gene of 74 FCoV strains was analysed. Phylogenetic analysis was carried out using the maximum likelihood approach and a variety of statistical analyses regarding nucleotide diversity were performed. Datamonkey analysis was carried out to determine whether particular sites in the N gene were subjected to positive selection.

Results: Phylogeny showed a general clustering trend on the basis of geographic origin rather than on virulence characteristics, but it also showed a strict correlation between some avirulent strains detected in chronic carrier cats and virulent strains, despite their different geographic origins. Furthermore, the analysis of the pattern of nucleotide substitutions, has evidenced "hot spots" sites subjected to selective pressure which may represent immunological domains.

Discussion: These findings support the prevalent role played by host and enviroment factors compared to the intrinsic viral characteristics in affecting the course of FCoV infection. Finally, in light of the potential immunogenic role of the N protein, N sequences data could be used in the future to develop an effective and innovative vaccine.

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[A.5]

Pistacia lentiscus extracts as biological products to control gastro-intestinal nematodes

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Gastro-intestinal nematode parasitism in grazing ruminants is a serious problem worldwide. Failure to control gastro-intestinal nematodes (GIN) results in poor growth rates, ill-thrift and, sometimes, death of animals. The predominant GIN implicated are *Teladorsagia circumcincta* and *Trichostrongylus spp*. *Haemonchus contortus* and *Oesophagostomum spp*. Nematodes have developed resistance to a wide array of chemical anthelmintic drugs, and consumer perception of chemical treatment is negative. Several *in vitro* and *in vivo* studies have shown that tannins and phenols have anthelmintic effects in ruminants. The mode of action of tannins on GIN is direct, with evidence that plant tannins impair larval development and viability. We assessed the anthelmintic value of two brush species that dominate the Carmel Heights of Israel; i.e. *Pistacia lentiscus* and *Phillyrea latifolia*. Leaf chemistry of *P. lentiscus*, but not *P. latifolia* is characterized by an exceptionally high concentration (20% in dry matter) of poly(ethylene)-glycol-binding tannins. Phenols and tannins were extracted from both plant species with ethanol 70%, 100% (v/v), or boiling water. L3 larvae hatched from eggs collected from a goat flock were incubated with the plant extracts before they were subjected to the exsheathment test. Ethanol 70% extract of *P. lentiscus* resulted in complete inhibition of exsheathment at concentrations that are prevalent in the gastro-intestinal tract. The efficacy of inhibition by ethanol 70% extracts was high, compared to 100% ethanol or water. Extracts of *P. latifolia* had minor effects. Our results suggest that tannins from *P. lentiscus* impair the ability of infecting larvae to develop into adults. This is in agreement with our observation that goats kept on a shrubland dominated by *P. lentiscus* have very low levels of helminth egg excretion. Our finding that extracts of *P. lentiscus* are more potent anthelmintics than extracts from other Mediterranean plant species is consistent with the findings that most polyphenols in *P. lentiscus* (Ethanol 70%) are galloyl derivatives of high molecular weight, and galloyl derivatives of catechin prevent exsheathment of the GIN larvae.

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