

9 Statistical power calculations. R. Lenth*, *University of Iowa, Iowa City.*

The talk will focus on how to do meaningful power calculations and sample-size determination for common study designs. There are important guiding principles: (1) Certain types of retrospective power calculations should be avoided, as they add no new information to an analysis. (2) Effect size should be specified on the actual scale of measurement, not on a standardized scale. (3)

Rarely can a definitive study be done without first doing a pilot study. Sample-size guidelines for pilot studies will also be briefly discussed. Finally, I will present some examples, using Java applets that I have developed and that are available on the web at <http://www.stat.uiowa.edu/~rlenth/Power/>.

Key Words: Statistical power, Sample size

10 Procedures for statistical treatment of binomial and categorical data. R. Quaas*, *Cornell University, Ithaca, NY.*

The analysis of discrete observations has always presented problems with a variety of methods of varying complexity but typically none simple. Unless, that is, one ignores the categorical nature of the data and does a usual analysis of variance. Surprisingly this often does quite well yielding "p values" quite similar to more appropriate methods, e.g. logistic regression for binomial data. If one is interested in estimates, however, these may be more interpretable using an appropriate non-linear method. Currently there is readily available software for the analyses. A series of case studies will be presented.

Key Words: Categorical data, Binomial data

Triennial Reproduction Symposium: Symposium II — Reproductive Immunology

11 Regulation of immune cells in the uterus during pregnancy in ruminants. P. J. Hansen*, *University of Florida, Gainesville.*

Pregnancy results in a change in number and function of immune cells in utero that potentially affect fetal survival and uterine defense mechanisms postpartum. These changes are driven by local signals from the conceptus as well as from hormonal changes mediated by the placenta or maternal system. In sheep, for example, macrophages accumulate in the uterine endometrium during pregnancy. Use of a unilaterally-pregnant model, in which pregnancy is surgically confined to one uterine horn, has revealed that accumulation is due to both systemic signals (numbers of cells in the non-pregnant uterine horn of the unilaterally-pregnant ewe higher than amounts in uteri of non-pregnant ewes) and locally-produced signals (number of cells in the uterus of unilaterally-ligated ewes higher in the pregnant horn than in the non-pregnant horn). Gamma-delta T cells also accumulate in uterine epithelium during pregnancy as a result of unidentified systemic signals. These cells may participate in growth of the conceptus, immunosuppression, or placental detachment at parturition. One of the key regulators of uterine immune function is progesterone. In sheep, progesterone can block tissue graft rejection in utero when injected to achieve concentrations too low to directly inhibit lymphocyte proliferation. Progesterone probably inhibits uterine immune responses in sheep indirectly by inducing secretion of a member of the serine proteinase inhibitor family called uterine serpin from the endometrial epithelium. Uterine serpin can block lymphocyte proliferation in vitro in sheep and natural killer cell mediated abortion in vivo in mice. Uterine serpin is also present in cattle, goats and pigs but its role in immune function in these species has not been documented. The relevance of changes in uterine immune function to the reproductive and immune status of ruminants has not been established. There is little evidence for immunological causes of pregnancy loss (except for cloned fetuses) but the downregulation of uterine immune function during pregnancy is likely to lead to a postpartum uterus with compromised capacity for preventing establishment of infectious disease.

Key Words: Pregnancy, Ruminants, Immunology

12 Why is the fetal allograft not rejected? C. J. Davies*, *Washington State University, Pullman.*

In viviparous species the fetus must be protected from a potentially hostile maternal immune system. The major histocompatibility complex (MHC) is a genetic region that encodes MHC class I (MHC-I) and class II (MHC-II) proteins, which present peptide antigens to T lymphocytes and induce graft rejection. MHC-II proteins are only expressed on professional antigen presenting cells. However, classical MHC-I proteins are expressed on all nucleated somatic cells. Protection of the fetus from immune-mediated rejection involves down-regulation of classical MHC-I antigen expression on trophoblast cells, which form the external epithelial layer of the placenta, and maintenance of an immunologically favorable, immunosuppressive, environment in the uterus. Normally, bovine trophoblast cells do not express MHC-I antigens prior to day 120 of pregnancy. However, third trimester trophoblast cells in the interplacental and arcade regions of the placenta express both classical MHC-I proteins, that could potentially induce fetal rejection, and non-classical MHC-I proteins. A human non-classical MHC-I antigen, HLA-G, is an important immunoregulatory factor required for maintenance of pregnancy. In cattle, third trimester MHC-I expression has no adverse effects and probably contributes to placental separation at parturition. However, somatic cell nuclear transfer (SCNT) fetuses, the majority of which are aborted between days 30 and 90 of pregnancy, had trophoblast cell expression of MHC-I antigens prior to day 34 of pregnancy. In conjunction with increased trophoblast MHC-I expression, SCNT pregnancies exhibited a marked increase in the number of stromal lymphocytes in the uteri of surrogate dams. A retrospective study found that SCNT pregnancies established using MHC-I homozygous cell lines, where the immunological barrier is greatly reduced, had significantly improved fetal survival from day 28 to term (51% survival for MHC-I homozygous and 5% for MHC-I heterozygous SCNT fetuses). Consequently, it appears that the high rate of fetal mortality in SCNT pregnancies is due, at least in part, to inappropriate expression of trophoblast MHC-I antigens and immune-mediated placental rejection.

Key Words: Abortion, Immunology, Bovine

13 Seminal plasma signalling in the female reproductive tract. S. A. Robertson*, *The University of Adelaide, Adelaide SA, Australia.*

Providing sperm for conception is generally thought to be the sole male contribution to pregnancy. But this view is now outdated – as well as sperm, semen contains potent signalling molecules that influence female reproductive physiology to improve the chances of pregnancy success. Cytokines and prostaglandins secreted by seminal vesicle and prostate glands bind to receptors on target cells in the female reproductive tract, activating changes in gene expression leading to modifications in the cellular composition, structure and function of the cervix and uterus. The consequences are increased sperm survival, ‘conditioning’ of the female immune response to tolerate the conceptus, and molecular and cellular changes in the endometrium that facilitate embryo development and implantation. Male-female tract signalling occurs in rodents, livestock animals and all other mammals so far studied including humans. The key active factors in seminal plasma are identified as members of the transforming growth factor- β family.

Experiments in rodents show that disruption of seminal plasma priming causes fetal growth retardation and changes in placental structure, with long-term consequences for growth of the neonate. Recent studies indicate a similar function for seminal plasma in the pig. In gilts, seminal plasma elicits an endometrial response with recruitment of inflammatory leukocytes and induction of pro-inflammatory cytokines and cyclo-oxygenase-2. The consequences persist through the pre-implantation period with altered leukocyte populations and cytokine parameters seen for at least 9 days. Exposure to semen also alters the dynamics in pre-implantation embryo development with an increase in the number of embryos and in their viability. Furthermore seminal plasma influences the timing of ovulation, corpus luteum development and progesterone synthesis. A better understanding of seminal plasma signalling may lead to new treatment products and management protocols to ensure maximal fertility and reduce embryo mortality in livestock animals.

Key Words: Seminal plasma, Cytokines, Pregnancy

Dairy Foods: Advances in Cultured Foods

14 Historical overview of lactic cultures. R. Sellars*, *R. L. Sellars and Associates, Waukesha, WI.*

A historic overview of the culture problems in the cheese industry that initiated the need for lactic culture research will be briefly presented. This led to the first commercialization of lactic acid producing starter-cultures used by the European and North American cheese industries. Interactions between the commercial starter houses, academia, and the cheese industry that led to the development of the current service oriented industry will be highlighted. The search for suitable cultures, protective media, plant sanitation procedures and starter rooms that reduce bacteriophage infections will also be discussed. The most important commercial milestones of starter-culture developments by commercial companies from 1878 to present and the strategies to produce flavorful, consistent hard cheeses will also be presented.

Key Words: Lactic culture, Cheese, Starter

15 Non-starter lactic acid bacteria. T. M. Cogan* and T. P. Beresford, *Moorepark Food Research Centre Teagasc, Fermoy, Ireland.*

In cultured dairy products, non-starter lactic acid bacteria (NSLAB) are only important in ripening and ripened cheeses. They mainly comprise facultatively homofermentative lactobacilli, e.g., *Lactobacillus casei*, *Lb. paracasei*, *Lb. curvatus* and *Lb. plantarum*, although pediococci and enterococci are found in lower numbers in some cheeses. Some obligate heterofermentative species, e.g., *Lb. brevis* are occasionally found. During ripening the NSLAB grow from low numbers of c. 10^2 to 10^8 cfu/g over several weeks or months depending on the cheese and its ripening temperature. NSLAB require an energy source for growth but lactose has been metabolised completely to lactate by the time exponential growth of NSLAB occurs. Citrate, arginine, sugars in lysed starter cells and in the milk fat globule membrane have been suggested as energy sources in cheese. The chromosome of at least one strain has been completely sequenced. Generally it is believed that their source is post pasteurisation contamination from equipment and air. However, many strains resist pasteurisation implying that raw milk is also a major source. The effect of NSLAB on cheese flavour has

been studied for over 120 years with variable results. More recent studies have generally shown a positive effect of selected strains on cheese flavour formation. During ripening, they are responsible for converting L lactate to D lactate and cause development of white spots on aged Cheddar cheese due to calcium-D-lactate formation. Many NSLAB metabolise citrate to formate and acetoin; they also metabolise amino acids, especially methionine to potent S containing flavour compounds like methanethiol and its degradation compounds, as well as various S containing esters. These and other aspects of NSLAB will be discussed in the presentation.

Key Words: NSLAB, Cheese, Lactobacilli

16 Insights from genomic studies on dairy lactic acid bacteria. J. L. Steele*, *University of Wisconsin, Madison.*

The genome of lactic acid bacteria (LAB) contains both plasmid and chromosomal DNA. The characterization of plasmids in lactic acid bacteria has been an ongoing area of study for the past thirty years. Characterization of LAB chromosomes begun in the early 1970s, however the most exciting developments in LAB genomics are now being fueled by nucleotide sequence information for complete chromosomes. Currently, the genome sequence is known or is being determined for more than twenty LAB. The value of genome sequence information for dairy-related LAB cannot be overstated. The availability of genomic sequences allows researchers to rapidly ascertain the genetic potential of an organism. For example, investigations into the proteolytic enzyme system of *Lactobacillus helveticus* CNRZ32 over a twelve year period had resulted in the identification of twelve genes encoding components of this system. However, within three months of obtaining a draft genome sequence of this organism, an additional thirteen genes encoding proteolytic enzymes were identified. Additionally, the availability of multiple genome sequences within a species allows for the study of strain specific traits. For example, a comparison of the complete genome sequence of two strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* identified regions involved in bacteriophage resistance, a trait known to vary from strain to strain. The availability of genome sequences also allows studies to follow