The Status of Laboratory Animal Production and Visions in the 21st Century
- Review -

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ABSTRACT: Today, laboratory animal production has decreased world-wide to half the number estimated in 1970 of more than 100 Mio. This is due to the cell-biological assays which replaced animal experimentation as a first allround method to solve biomedical problems. Animal experimentation remains the most significant experimental method for the study of higher organized physiological systems and their multifactorial connections. This requires maximal uniformity of all quantitative traits among the animals used for such studies (mainly mice and rats) and stability of these traits for reproducing such studies at any time world-wide. The success of the developed methods for the standardization of laboratory animals was analyzed and were found only partly be acceptable. Getting a higher degree of uniformity among standardized inbred animals is blocked by "intangible variance". This is caused by influences of ooplasm, shown by experimental twin and clone studies. Manipulation of this component of variance is essential in the future. Genetic drifts impair the necessary stability of biological traits. There are a few disadvantages associated with the cryopreservation of embryos and other methods are required. - Dogs and cats were replaced by pigs as laboratory animals. A new line of animal production will evolve over the next 25 years with similarities to the present laboratory animal production, because in future pigs were used as donors for xenotransplants for men. (Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 7 : 1142-1151)

Key Words: Uniformity of Twins, Cloning of Cattle, Intangible Variance, Individuality, Cryopreservation of Embryo, Pigs for Xenotransplantation

INTRODUCTION

The breeding and production of laboratory animals has a short history of around 100 years. A short time when compared with the 10,000 years history of breeding and raising farm animals. Basic sciences in medicine and biology originated around 300 years ago. However, over the first 200 years, only few experimental studies on animals have been carried out to provide answers to these scientific questions. During the 18th and 19th centuries, animals were not bred for experimental purposes. At the end of the 19th century, basic knowledge became a higher significance for clinical medicine. From this time on, animal were bred and produced for research. Paul Ehlich was one of the first to breed guinea pigs in his Frankfurt laboratory in the 80s of last century. These were produced mainly for medical research and for the diagnosis of infectious diseases. Donaldson (1924) reported on the initial breeding of rats in 1893 in the USA. Breeding mice and rats especially for genetic studies, began at the end of the 19th century and the beginning of the 20th century in the USA, France, Great Britain, Switzerland and other countries.

In my paper I shall restrict information on warm blooded vertebrate species and on the following five topics: (1) Quantitative and qualitative trends of laboratory animal production during this century. (2) The limited success of the efforts to standardize laboratory animals. (3) Task of the future: Looking for biological reasons of intangible variance (peristent part of random variability) and for ways to manipulate it. (4) Stability of the biological characteristics of laboratory animals over time now and in future. (5) Pigs are mostly used as larger laboratory animals now and in future. Pigs used for xenotransplantation?

QUANTITATIVE AND QUALITATIVE TRENDS IN LABORATORY ANIMALS REQUIRED THROUGHOUT THE CENTURY

The number of laboratory animals raised per annum worldwide is yet unknown. Only approximate evaluations are given for some countries by the breeders or informed persons. Garstline (1963) reported around 27 million mice and rats bred in the USA to meet with the demands in 1957. This number increased within 12 years, up until 1969, to about 60 million. In 1970, a yearly production was assumed of about 12 to 15 million laboratory animals in Germany (Gärtnert 1991).
More information concerning animal production can be drawn from statistics on animal experimentation. Home Office in Great Britain published since 100 years statistical information on experimentation on a yearly basis. As from 1985, other European countries collected and published similar numbers on request from the European Community. These documented numbers, however, concern only the number of animals used for experiments involving pain, harm or distress. In spite of this restriction, these numbers can be helpful in obtaining estimates on the production and breeding of laboratory animals. From personal as well as other experiences, the number of bred laboratory animals is about two to three times higher then the number of animals reported to be used for experiments (table 1). The higher number of bred animals includes animals killed for obtaining isolated organs or tissue for research, breeding experiments, perpetuating genetic information such as mutants by breeding nuclei etc. Finally, the higher number results from overproduction in order to have available animals of specific ages, strains, sex, and for special purposes.

Of particular interest for future speculation are the quantitative changes over the past century of laboratory animals needed. Since 1890, the British Home Office has published yearly the number of vertebrates which were used for experimentation (figure 1). The changes shown are similar for the breeding and production of laboratory animals all over the world. Comparison between Britain, the USA, Germany, the Netherlands, Japan and other countries confirm that assumption.

The number of animals used in experiments has grown slowly and consistently from 1900 to 1950. However, after 1950 the growth rate increased exponentially over the following 20 years. Around 1970, the explosion reached its peak, characterized by a four-fold increase this year. After 1970, a remarkable reduction was seen over the ensuing 15-20 years. At the end of the 80s, a new plateau was

![Figure 1. Vertebrates used for experimentation in Great Britain (Home Office, 1941, 1971, 1994)]

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Table 1. Vertebrates used in (painful and distressing) experiments per year

Home Office (1941)
Home Office (1971)
Home Office (1994)
Gärtner (1991)
Gärtner (1991)
BML (1997)
BML (1997)
Zutphen (1995)
Zutphen (1995)
Kaegler (1995)
Kaegler (1995)
Lab.anim.Res.NASc
Zutphen (1995)
found of about two thirds of the peak level. This was also apparent in other countries worldwide.

The change in the need of laboratory animals comes from the time limited use of animal experimentation as the main method in most of the disciplines. Only in a few disciplines animal experimentation is the main method of research and permanently used. The most prominent example for such a discipline is surgery-research. But there are many disciplines where animal experimentation is only the initial method to gain scientific understanding of the new biological or pathobiological field of such disciplines. Figure 1 reveals the periods when such disciplines of life sciences executed their research predominantly by animal experimentation. After 20-50 years working with animal experimentation, the biological understanding of the phenomenon of interest of those disciplines gave detailed knowledge which induced supplementation of the first allround method. At that time, the preferred methods were those restricted to the details of the biological phenomenon of interest. Methods changed to isolated systems which are more precise for detailed examinations.

About 60 years ago, bacteriology changed from animal experimentation to isolated studies by developing highly sophisticated cultures for selective breeding of particular germs using biochemical and immunological methods for diagnostics and other purposes. Some years later, virology changed from studies in total animals to cell cultures, the use of the electron microscope, immunological methods etc. Again, years later, pharmacology changed from studies in total animals to studies in isolated organs and cells cultures. This was supported by molecular-biological knowledge, which later became actuality in all fields of life sciences.

The peak of laboratory animals used between 1950 and 1990 was based on the growing fear upon chemical and radiological hazards. Toxicology, oncology and safety studies for public health became increasingly important. Acute, subacute and chronic toxicity tests were initially performed in animals studies. 20 years later the reduction in the number of animals used in such experiments resulted in the better quality of animals, higher standards applied for such studies, developed methods to judge them and, mainly, accumulated knowledge in cell physiology, biochemistry and molecular-biology, which now occupies all fields of life sciences.

For pharmacological and toxicological studies and for drug research screening purposes, animal experimentation was no longer the only way how to start toxicological investigations of a new compound. Studies with isolated cell and cell-physiological examinations became the first step to test in a simple assay the biological significance of new compounds.

Animal experimentation today is restricted to studies following in depth knowledge of isolated systems and the mechanisms on a cell-physiological and molecular-biological level. Animal experimentation today is needed for investigation on a higher level of biological organisation i.e. on the level of organ and system interactions etc. This is of great significance in immunology.

For the different uses of laboratory animals, particular species and qualitative requirements became more and more significant and, therefore, influenced the production and breeding of these animals. Similar to farm animals and other animals used for experiments, laboratory animals also have to be appropriated to their use. But the aims of appropriation for laboratory animals are different from farm animals. Laboratory animals are needed for quantitative comparisons. Quantitative physiological or morphological traits of laboratory animals were estimated and the influence of experimental conditions on such traits studied. It is often necessary to make repeated comparisons using the same animals under changed conditions, at different times, and in other locations around the world.

Therefore, principally two different requirements of laboratory animals have to be fulfilled by laboratory animal production. Firstly, high uniformity of morphological and physiological characteristics should be realized among the animals used for experimental studies. High uniformity improves the comparison of control and experimental groups. Secondly, stability of such characteristics over time and in different generations of the animal should be realized. This is demanded for reproducing experimental studies on other locations in the world and at other times. Breeding, production and husbandry of most laboratory species aims on improving uniformity and stability of biological characteristics.

Both requirements are only partly covered until now in spite of the many efforts undertaken in the last 60 years. I should like to reasoning for this and in order to speculate future goals of laboratory animal production.

**HIGH UNIFORMITY AMONG LABORATORY ANIMAL: ACTIVITIES FOR REDUCTION OF THE DEVIATION OF BIOLOGICAL TRAITS AND ITS SUCCESS**

There are various papers which describe these activities in detail. Therefore, I would like to comment briefly only on the topics of laboratory animal standardisation.

Elimination of the genetic variability has the longest tradition. The most successful way is inbreeding. Inbreeding for more than 100 generations
can result in homogeneity on all autosomal gene loci and in isogenicity among the animals of an inbred strain. Different tests and permanent control of those strains, including molecular-genetic analyses, verify that outcome. Uniformity of the genotypes can realized also in F1-hybrids by crossings of defined inbred strains.

To standardize the environment, the laboratory animals are subjected to specific living conditions. Synthetic or semi-synthetic diets are calculated on the base of nutrient requirements, its components were standardized and freed from any unknown products. - Germ interactions and all infection were eliminated which could greatly disturb longterm animal experimentation.

This is performed by caesarian-derived, germfree animals which are further bred and studied in isolators, or by germfree animals contaminated with a gnotobiotic or an infection-resistant microbiological flora, or by animals free from microbiological pathogen known as SPF-animals. All such animals have to live behind barriers and are under constant control.

Husbandry standardization is realized by full climatization of the animal rooms with standardized lighting to synchronize the biological clocks of the animals. We endeavoured to provide the animals with an optimal social environment and a technically desig of the cages. This can be done by examining endocrine, circulatory, and behavioural stress parameters in such animals.

All these efforts are high-sophisticated and cost intensive. This has brought about remarkable changes in the commercial production of laboratory animals and small private breeders were unable to keep up with the increasing quality demand in the 60s. The larger breeders adopted to the high conditions, and prices for the animals increase a tenfold. But, these breeders gave up in the 80s due to the decrease in number of animals needed. State-owned enterprises ceased to exist due to unprofitability. i.e. England, Germany, The Netherlands, Scandinavia. At the present time, world-wide coordination has resulted in a few commercial and governmental laboratory animal producers.

THE LIMITED SUCCESS OF THE EFFORTS TO STANDARDIZE LABORATORY ANIMALS

How successful have all the done efforts for reducing the deviation in morphological and functional traits of laboratory animals? The following study provides an answer.

Two types of variability influence the morphological and physiological traits present in laboratory animals: Fixed effects and random variability. Both are shown in figure 2 (Gärtner, 1990). On the left, the ranges of kidney weights are shown of 1450 male or female rats taken from 58 groups, each group consisting of about 25 animals. Each line represents the range of variability within a group of highly standardized animals. The groups differ in type of standardization. They differ in genotype (inbred strains F1- or F2-hybrids), sex, age, in different standards of environment i.e. living under highly standardized environmental conditions, or living in the wild. A few groups were germfree, most of them of high SPF-

Figure 2. (Left) - Showing the influence of fixed effects: Ranges of the kidney weights in (gram) in 58 groups of male or female rats, inbred, F1-, F2-hybrids, living under highly standardized conditions in the animal house (black points) or living in the wild (open cycles), free form pathogens or contaminated by M. pulmonis (*). (Right) Showing random variability: Coefficients of variation multiplied by 4. (for details see text)
quality, others conventional, and a few contaminated with mycoplasma pulmonis.

**FIXED EFFECTS CAUSING BIOLOGICAL VARIABILITY**

The left figure of figure 2 shows many fixed effects among the 1450 animals. Fixed effects are realized by the similar location of the distribution of the single values of a group and of a few "related" group in the total range. The deviations of "related" groups clotted together. Different clods are frequently polar distributed in the distribution of all. The clods are caused by fixed effects, e.g. identities or similarities in sex, age, or the genotypes of the animals, or by their living conditions, state of health or biotic and abiotic environmental conditions.

One of the aims of laboratory animal standardization practise is to provide optimal fixed conditions for the animals. By this way variability caused by the influence of different fixing effects can successfully omitted. By this way the performance of chronic studies in many disciplines became realization.

**RANDOM VARIABILITY CAUSING BIOLOGICAL VARIABILITY**

Another aim of laboratory animal standardization practise is the reduction of the random variability. Random variability refers to the range of quantitative morphological and physiological characteristics in spite of standardization which remain within a group of highly standardized inbred animals. This range frequently resembles a Gaussian distribution.

For an improved study of random variability e.g. for kidney weight, the fixed effects were omitted by calculating the coefficients of variation for each group ((standard deviation/mean) x 100) i.e. standard deviation expressed in percentage of the mean. On the right hand side of figure 2, each single lines represents the coefficient of variation (multiplied by a factor of 4) for each of the 58 groups of examined rats. This multiplication gives a more realistic figure of the phenotypical range. For comparison, all the lines are put in order of magnitude. The range (shown left side in figure 2) and the coefficient of variation (right) lying on the same plateau comes from the same group.

In 49 of the 58 groups, random variability of the kidney weight showed no significant difference between the groups. In the remaining nine groups the range was significantly larger. Animals in these groups were infected with mycoplasma pulmonis. Infections are a very potent source for enhancing random variability. This is the same for all the other 24 examined physiological and morphological traits investigated in these rats, but not shown here. All the other various effects of standardization of the genotypical differences or environmental conditions showed only very little influence on the range of the random variability of a designated group. The reduction of the genetic variability by using inbred animals instead of F2-hybrids or outbred animals did not significantly influence the random variability (figure 2 line right). Standardization of different environmental components such as food, temperature, group size, bedding, humidity, germ-free or SPF conditions does not reduce the random variability. This was also true when the coefficients of variation were compared between animals living under highly standardized laboratory conditions in an animal house and healthy animals living for over five months under variable wild conditions. The latter group lived in a confirmed area of about 200 m² in the forest from February to October and were fed a varied diet twice a week. They sometimes lived under overcrowded social conditions because of unrestricted reproduction. Surprisingly, the enormous increase of environmental variability under wild conditions did not increase the range of random variability.

These results (Gärtnner, 1990) show that manifold small environmental noises, which were for long time supposed to be responsible for random variability in inbred strains, do not play a major role as a source of random variability. Extreme standardization practise has only a minor influence on the variability of physiological and morphological quantitative characteristics. A large part of random variability in inbred mice and rats resists all attempts toward standardization (Gärtnner, 1991). It qualifies as an intangible variance. Wright (1933) and Falconer (1960) and others announced this component of variance as "intangible" when they first demonstrated and described it about 70 years ago.

From this results we can conclude that the genetic and environmental standardisation practise for laboratory animal only effects in the successful achievement at fixed conditions and by this it eliminates the part of variability which is caused by different fixed effect only. However, random variability resists such standardisation efforts.

Therefore, the question requires an answer, namely, what are the reasons for the intangible variance which resists all efforts of standardisation?

**TASK OF THE FUTURE: LOOKING FOR BIOLOGICAL REASONS OF INTANGIBLE VARIANCE (PERSISTENT PART OF RANDOM VARIABILITY) AND WAYS TO MANIPULATE IT**

One of the aims of laboratory animal production in future will be the further improvement of uniformity of the animals needed in life sciences. Therefore, I
want to summarise recent results (Gartner et al., 1998) which may help to answer the question regarding the biological reasons for that large component of random variability which resists all attempts toward standardisation and qualifies as an intangible variance. Different modulation of the gene functions caused by ooplasmic influences and effective at the earliest stage of development are suggested to be the reasons for the intangible variance. They are different for each individual. These suggestions come from studies in experimental twin research and such in cloned animals. This was done in cattle and mice. At first I summarise the cattle results.

Figure 3 (middle) depicts the stages in the development of cattle. The individual development of the shown three calves begins at fertilisation of an oocyte and is followed by the two cell-stage, other cleavage stages, the morula stage, and, the many developmental stages before and after birth. At the bottom the variability of the body sizes of many born and growing calves are shown, identical in sex and age.

Figure 3 (left), compares the steps of biological development and the final differences among the animals (shown in figure 3, middle) with a mechanical model, the nail board of Wallace, the first twin-researcher and a cousin of Darwin, which he applied for explaining random distributions. It shows the different locations of identical balls after crossing a nail board, resembling a Gaussian-distribution. The model depicts how that typ of variability may originates by a system of progressing steps, each step biased by small disturbances.

In order to detect the steps which are mainly responsible for the final variability, repeated starts at those steps have to be done and their effects on variability can examined. Repeated attempts at two different stages of the early development of calves were undertaken: At first, at the earliest developmental stage, immediately after fertilisation, at the one-cell-stage. This is done by cloning. Secondly, later at the 8-cell stage. This is done by examination of naturally born monozygotic twins which results from spontaneous splitting of blastocysts or of artificial twins prepared by splitting one blastocyst followed by transfer of both halves into different surrogate mothers. In animals derived from preparations at both, different developmental steps, many characteristics were measured and the variability within couples and among all animals compared (Gartner et al., 1998, Gärtnner et al., 1991).

Figure 3, right, summarises on both nail boards the results analysed for twelve different body traits measured. Within monozygotic twins only 5-20% of

Figure 3. Developmental steps responsible for interindividual variability inisogenic animals. Analyzed (right, below) by differences within monozygotic twins (<20% of total variance) and (right, above) by differences within cloned siblings (40-70% of total variance) in calves. Reference to ooplasmatic influences causing the intangible variance and determining the individuality.
Table 2. Components of variance of the body weight in monozygotic (MT)-, dizygotic (DT)- pairs and NB-sisters

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<th>N1/N2</th>
<th>body weight</th>
<th>$S_i^2$</th>
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<td>8/4</td>
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<td>0.31</td>
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<td>95%</td>
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<td>1.41</td>
<td>0.97</td>
<td>68%</td>
<td>0.44</td>
<td>31%</td>
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The total variance exists, this is the well known uniformity of monozygotic twins assessed in man, cattle, sheep, mice, and armadillos. Nearly the same close similarity is true if the twin mates, after splitting of their common blastocyst, were transferred to two different foster mothers. This experiment was carried out in cattle. It revealed that only minimal influences for determining individuality occur after the 8-cell stage and come from environmental influences. But common signals active before splitting affects their high uniformity.

That becomes clear, by repeated starts at the earliest stage of development at the one-cell-stage. Repeated starts were performed, at the initial contact between the new formed nuclear genom of an individual and the surrounding ooplasm. Such repeated starts were done by experimental cloning.

Artificial cloning is performed by separation of the blastomeres in the 8 to 32-cell stage, followed by transplantation of each separated blastomere in an unfertilised and enucleated oocyte from the same or a different mother. So, identical nuclear genoms of cloned siblings develop independently from each other. Each new embryo is transplanted in a different foster mother who then carries it and gives birth to it. We were able to obtain results from cloned cattle, the only species in which until now cloning could be successfully performed on a large scale (Gärtnér and Bondioli, 1998). On comparison of different characteristics in the cloned calves three months after birth, the variance within the clones revealed an astonishing high percentage of 40-70% of the total variance (figure 1, right), which is much higher and significantly different from those revealed by pairs of monozygotic twins.

Comparison of the two variances obtained from monozygotic twins in cattle and those obtained from cloned calves demonstrates that between the start of development and the 8-cell stage, a high degree of individuality is determined. The starting process of development of an individual, induced by contact between the defined nuclear genom and the surrounding ooplasm with its organelle does not strictly follow the path of development determined by the genom. Ooplasmatic constitution, ooplasmic mitochondria, or conditions described in chaos theory (Cramer, 1989) play an influential part in determining the individual.

These results revealed a component of variability brought about by ooplasmatic signals and independently from nuclear genome and the environment. Therefore, three components determine individuality: genom, ooplasmatic signals and environment. The component of variability which is caused by ooplana influence resists all attempts toward its manipulation it is qualified as an intangible variance.

The natural occurrence of such a typ of variance were also shown in twin studies performed on inbred mice (Gärtnér & Baumack, 1981). Inbred mice or rats are genetically identical. Therefore, litter mates of inbred animals are cloned animals, but produced by a traditional breeding process. To answer the question whether or not the large differences within litter mates in inbred animals are created also at a very early developmental stage, experimental twin studies were performed. Blastocysts of the 8-cell stage of inbred mice were split, both halves were transferred to a foster mother who then carries and gives birth to them. In the adult mice, many quantitative traits were estimated and compared within monozygotic twin mates (MT), within naturally born siblings (NB) and within dizygotic twin mates (DT).

Table 2 depicts some results obtained in two different inbred strains, C57BL and AKR. Total variance ($s^2$) and its components within ($s_o^2$) and between ($s_b^2$) the twin mates and sister pairs. Very small differences within the MT- mates are obvious. These differences are responsible for 5, 12 or 30% of the total variance. The 5% and 12% are similar to those found within MTs in cattle, and known from other species including man. However, in contrary to
DTs and NBs the component between (S<sub>5</sub>) in MT are largest. This is the large variability which we know from differences within cloned siblings. The MT-twin pairs are situated closely together in the wide range of that variability and both closely together. Both mates of such a MT-pair received an identical ooplasmic signal to determine the body sizes of the adults before splitting of their common blastocyst. The differences between the MT-pairs corresponds with the differences within DT-twins or NB-pairs, where each member got its own ooplasmic signal.

Such twin studies in inbred mice clearly show that intangible variance is originated at the very early stage of the development of an individual and the decided component of random variability in laboratory animals.

I wish to point out from this report that intangible variance and its manipulation is one of the future tasks in order to improve the appropriation of laboratory animals. However, due to my own experience it seems very doubtful, that production of monzygotic twins is a realistic path to take.

**STABILITY OF THE BIOLOGICAL
CHARACTERISTICS OF LABORATORY
ANIMALS OVER TIME, DIFFERENT
GENERATIONS AND LOCATIONS**

I now wish to discuss the second general requirement for the production of laboratory animals: Stability of the biological characteristics of laboratory animals over time, different generations and locations. It results from the necessity of researchers to use the same animals under changed conditions, at different times, and in other places around the world. This requirement is important because, as mentioned above, different fixed effects influence research results tremendously. Differences between genotype have a very effective fixation power.

On the other hand, the genotype differences among strains of laboratory rats and mice are vast. This is caused by the ability of mice and rats to adapt quickly at changed environmental niches due to their high reproduction rate and short generation time. It is also caused by the breeding strategy applied to this species for producing laboratory animals. Breeding started about 100 years ago from wild or semi-wild ancestors. In laboratory animals the sole breeding goals were tameness, reproduction success, and in a few strains, fixation on an isogenic genotype. Such breeding strategies differ from that of farm animals, which always had a more closer aim, e.g. increase of milk production, increase in body weight etc. This deficiency of a close breeding aim and the desired inbreeding direction creates a wide ranges of functional and anatomical diversities among the many strains and stocks of laboratory animal. Their number increased during the last century tremendously. Festing (1993) reports and describes to day more than 400 well defined mice and more than 250 rat inbred strains, more than 1000 mutants which are maintained in thousands of stocks around the world. Fifty years ago only 10% of this amount existed. Nowadays, the attained number has become an extremely enlargement by the production of transgenic mutants, by creating coisogenic lines and others.

Laboratory animal production today is confronted with the user's demand to preserve the particular biological quality of each of the very different strains and stocks in its actual originality. This is a difficult task. Each inbred strain and many more outbred stocks have succumbed to genetic drifting. Within 6-10 generations, the quality drifts are remarkable in inbred animals. This was frequently recognized between sublines of inbred strains.

**CRYOPRESERVATION AND REVITALISATION**

Reducing that power of divergence during the breeding progress and preserving the particular biological quality of a laboratory animals genotype over time and generations is another large task of laboratory animal production for to day and the future. Cryopreservation of embryos and their revitalisation after a certain time for backup starts of an inbred nucleus is considered to day the only possible technique to maintain an inbred strain genetically constant over a long period of time. Many methods for cryopreservation of embryos have been development in the last 30 years and their application in laboratory animal production are presently being tested.

Beside this purpose freezing preimplantation embryos is a proper method in reducing the costs and space for maintaining the multiplicity of strains of mice and rats in vital colonies, and in allowing for eradication of infections if embryo transfer is performed under aseptic condition into barrier maintained surrogate dams.

Many difficulties for this method comes from the impaired success of revitalisation of thawed embryos and of embryo transfer to foster mothers which give birth to them. The results differ remarkably between strains. An average revitalisation rate of 20% is assumed (Hedrich, 1990). If embryos are obtained from normal donor females, there is a minimal chance of revitalising one fertile brother-sister pair needed as a prospective breeding nucleus of an inbred strain. Therefore, superovulation is performed to enhance the
number of embryos per mother. Until now more than 100 cryopreserved embryos are regarded as inferior limit to provide a safe backup of a strain (Mossmann et al., 1998). With an effective superovulation, 10-20 two-cell embryos per mother should be disposable in rats and mice. But only 30% of treated females respond on the treatment in such a manner. All the embryos of a successful mother are collected together in one batch. And for successful preservation of a strain 10-20 batches per strain should be stored deep frozen at less than -80°C.

**EMBRYO CRYOPRESERVATION AND GENETIC CONSTANCY**

In spite of the mentioned disadvantages, cryopreservation of embryos is today considered to be the best technique in order to maintain genetic constancy. However, the question arises whether or not storage of embryos under freezing conditions results in an increase of the spontaneous mutation rate.

The mutation frequency/locus and generation has been estimated in vital mice with $6.6 \times 10^3$ (Russel and Russel, 1996). Does it increase?

Mobraaten and Bailey (1987) performed tail skin transplantation in 4000 descendants of revitalized embryos and did not find any deviation. Dorsch and Hedrich (1998) tested more than 200 gene loci (immunological and biochemical makers, results from DNA fingerprinting etc.) in around 2500 descendants of revitalized embryos from different inbred strains without finding any deviation. Both results show that the mutation rate does not remarkable increase. In addition, Whittingham et al. (1977) and Lyon (1976) revealed no reduction in viability of embryos after exposure of the cryocontainer to an increased long-term background irradiation. It was assumed that the effects of background irradiation on the induction of mutations would be only marginal. However, recent reports describe mutagenic effects due to the complete cycle of cryopreservation (Bonquet and Aurox, 1993; Dulionst et al., 1995). These authors described chromosome aberrations and/or DNA damages. But DMSO was used as a cryoprotectant which is known to be embryotoxic. From those findings was it concluded that cryopreservation would be of no, or only of a small genetic risk. Further studies are required to estimate the real mutation rate in larger groups of animals.

There is a rapid success in the field of reproduction biology induced by gentechnology. This may bring about better procedures for fulfilling the demands for genetic constancy in laboratory animal production over the next twenty years (primordial, embryonal stem cell, nucleus transplantation etc.)

**PIGS ARE MOSTLY USED AS LARGER LABORATORY ANIMALS NOW AND IN FUTURE. DOES PIGS BECOME DONORS OF XENOTRANPLANTS FOR MEN?**

Most of the information given in that paper was pointed on mice and rats. It has also good relations to guinea pigs and rabbits but only small for larger laboratory animals as dogs, cats, sheep, pigs and monkeys. At the end of my paper I want to mention changes among these larger laboratory animal species. Primarily animal welfare considerations and pressures led to the replacement of dogs, cats and monkeys. Breeding mini pigs of different stocks were performed in some countries. That type of pigs grow slowly and stay on a body weight of around 50 kg over a few years. Until now they differ remarkably in their outfit and health stage. But such animals replaced successfully the use of dogs in long term toxicological and clinical experimentation, above all cardiological and transplantation research. On the other hand sheep replaced dogs more and more in bone-surgery studies. Twenty years ago a special stock of sheep were bred descending from African naked sheep in order to fit with the indoor husbandry of laboratory animals. But these animals were not accepted. Bone studies also chronic heart-surgery studies in sheep were done and the animals lived after recovery outdoors.

Finally a new branch of the use of animals in medicine stands for our doors: Xenotransplantation and the care of animals which will become the donors for kidney-, liver- and other organs transplantats to human patients. Pigs are the most probable species for this purpose. Transgenic pig strains are in preparation carrying human derived signals which abolish the very acute and special repulsive reaction of xenotransplants. Production of such animals will be done under patent protection in special industrial drug companies but after buying them they have to be stored under special hygiene conditions near the transplantation hospitals. Control and special husbandry of such pigs will become a special task of veterinarians in future.

**REFERENCES**


Festing, M. 1993. International Index of Laboratory Animals. Lion Litho Ltd. Carshalton, Surry, GB.