

Breeding of laboratory mice for biomedical research

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Mice are because of their genetic similarity to humans, short reproductive cycle, short life span, small size and relatively low cost of maintenance one of the most frequently used animal models for biomedical research. Despite the dominant trend in reducing the number of animals used in biomedical research, we should not ignore the fact that many of the medical benefits have been discovered through the work on laboratory animals. It has been widely accepted that animal experimentation should be performed only when no alternative is available, and given that all measures are taken to preserve the welfare of laboratory animals and minimize discomfort. Mice are nocturnal, burrowing, climbing and social animals with anatomical and physiological characteristics of rodents. Mice are usually breed and housed in polycarbonate cages, preferably in ventilated racks. The present review describes husbandry and mice handling which allows normal behaviour, feeding, breeding and growth. We suggest a possibility to reduce the number of animals needed in the research of Diabetes mellitus by using freshly prepared pancreatic slices. This is a novel isolated tissue preparation for electrophysiological and imaging studies on beta cells and other cell types in the pancreas and other tissues.

Key words: mouse breeding, husbandry, animal welfare, identification

INTRODUCTION

Mice are as laboratory animals the most widely used vertebrate species from the order *Rodentia.* Majority of them belong to the genus *Mus*. The most commonly used mouse is *Mus musculus*, other mice used in research are *Mus spretus, Mus caroli, Mus pahari, Mus domesticus* and *Perumyscus spp* (Suckow et al. 2001). Because of short reproductive cycle, short life span, small size, genetic manipulation and relatively low cost of maintenance, mice are suitable models for biomedical research. The high degree of genetic similarity of mice to humans is another reason for being the experimental model of choice in research. According to European Biomedical Research Association 11 million animals were used in 2002 in EU for the scientific research. Rodents together with rabbits represent the majority of animals with 78 % (51 % of all animals were mice). The majority of animals were used for basic biological studies, for research, development and quality control of products and devices for human medicine and dentistry and for veterinary medicine, for toxicological and other safety evaluations, for diagnosis of disease and for the education and training (Communities 2005). The majority of laboratory animals (63 % of 12000) were mice, but they were mostly used for applicative research (Ministry of Agriculture 2007).

During the recent decades, civil initiatives appeared appealing against the use of animals for scientific research, with the standpoint that no experiments could be considered essential. However, majority of the population still recognized that many of the medical benefits have been discovered through the use of animals. Laboratory animal research still stays a fundamental principle to gain knowledge to assure public, animal and environmental safety and to improve public health. It has to be emphasized that animal experimentation can only be performed when no alternative is available and when the benefit of the experiment outweighs the animal's suffering. The use of laboratory animals obligate researchers to preserve their welfare and minimize discomfort (Kaliste 2004).

In considering alternatives to animal research subjects, the concept of "alternatives" called 3R (replacement, reduction, and refinement) was developed by Russell and Burch in 1959 (Russell and Burch 1959). Replacement of animal in research is substituting animals with less sentient animals or with nonanimal models. Replacement can be accomplished by replacing a warm-blooded animal with a cold-blooded animal. Other possibilities are computer simulations, tissue cultures and many other models. Reduction may be accomplished by reducing the numbers of animals used in the research. It can also involve organs and tissues taken at the animal's time of death to be used for research that does not involve whole animals. Refinement includes all aspects of veterinary care, efforts to minimize pain and distress via anaesthesia, analgesia, aseptic technique in surgery, husbandry details, and environmental enrichment. Studies in rats have shown that increased handling resulted in decreased stress during procedures (Chow et al. 2008).

CHOOSING THE RIGHT ANIMAL MODEL

Animals chosen must be appropriate species and quality to answer the scientific question. In general, breed animals are preferred to wildlife. Proper choice of animals minimize genotypic and phenotypic variables in the physiological response of the animals (Chow et al. 2008).

Inbreed mice – Mice of the particular inbreed strain result from a minimum of 20 successive generations of brother-sister matings and are virtually identical to all other mice of the same strain.

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Outbreed mice – This is breed to minimize inbreeding and ensure genetic variability. They are usually used in toxicology. As each animal is virtually a unique genetic individual, it is similar to testing a heterogeneous population. The animals have the disadvantage of being phenotypically variable and come with variable genetic make up.

F1 Hybrid mice – These mice are first generation crosses between 2 different inbreed strains. Each of these F1 hybrid mice is genetically as uniform as an inbreed strain.

Inbreed mice with a spontaneous mutation – These are inbreed mice that are perpetuated from a single mouse that was born with a significant genetic change. Spontaneous mutations have led to important advances in functional genomics. One of these are Ob/ob (leptin deficient), db/db (leptin receptor deficient) mice which led to the discovery of leptin and its role in body fat composition (Flint and Woolliams 2008).

Coisogenic strains – These are strains of mice that differ from each other only at one gene. The difference is due to the spontaneous mutation that occurred in that one gene. After the appearance of the mutation, these animals with the mutation are maintained as a separate strain from the original inbreed strain (Suckow et al. 2001).

Congenic mice – These mice are similar to the coisogenic mice except here the genetic dissimilarity is the result of the breeding rather than the result of a mutation.

Mice that carry induced mutations - Since human and mouse genomes are fully discovered we have an opportunity to use mutants as tools for the discovery of genes function and their role in diseases. In general two methods are used for the development of genetic mutation models; chemical mutagenesis and targeted transgenesis.

Chemical mutagenesis

The most simple way of causing mutations is treating mouse genome with chemical mutagens such as the alcylating agents N-ethyl-N-nitrosourea which causes point mutations in double stranded DNA. Treated mice have a 1000-fold increase in the rate of mutations which occur randomly in genomic DNA and because of that offsprings have to be screened for abnormalities. According to the literature (Flint and Woolliams 2008) over than 1000 strains of mice derived with these methods are currently used for functional genome studies.

Transgenesis and gene knockouts

Transgenesis is an introduction of additional genetic material to the genome with pronuclear injections where foreign DNA is injected into a fertilized egg, by viral transduction or transfection into embryonic stem (ES) cells. For selection of transgenic cells before transfer them into an embryo we can use genetic markers with a procedure called gene trapping. Another option when we want to study a gene function is a targeted deletion where genes can be selectively removed by homologues recombination with a mutated version of the gene. ES cells with mutated gene are then introduced into an adult mouse and breed to produce offspring homozygous for the desired deletion (Flint and Woolliams 2008).

Sex

Besides animal species, sex of the animals is also important when choosing the appropriate mouse for the research because it may cause experimental variability. Differences include hormone cycles, muscle:fat ratio, behavioural differences and variations in growth pattern.

ENVIRONMENTAL FACTORS

Environmental factors are important if we wish to fully control the variances of the physiological responses. We have to control the environmental conditions such as ventilation, noise, chemical agents, light/dark cycle and stress, in which the animals are breed and tested.

Ventilation

A good air exchange of 12-15 air changes per hour prevents disease spreading and keeps the concentration of waste gases to a minimum. Mice require the temperature of 19-25 °C and the humidity of 40-70 %.

Noise

Mice are very sensitive to ultrasound comparing to humans and care should be taken to reduce ultrasound in mice keeping facilities.

Chemicals

Ammonia is produced from bacteria breakdown of feaces and urine and it is toxic in high concentrations. Levels over 25 ppm can cause pathological changes in respiratory tract and cornea.

Light/dark cycle

Mice, like most species have a circadian rhythm and the duration, intensity and spectrum of light affect animal biorhythms (Flint and Woolliams 2008). Investigators should be aware that this may affect biological data and should standardize the time of day that samples/measurements are taken to avoid this affect. The standard light/dark cycle is 12/12h and the light intensity should not exceed 350 lux. This light cycle can be modified upon the request of the investigator in special circumstances.

Stress

To avoid consequences of stressed animals it is important to minimize the stress due to transportation, handling, overcrowding and isolation. Stress can depress immune system and thereby increase susceptibility to latent oncogenic and infectious agents.

ANATOMICAL AND PHYSIOLOGICAL FEATURES

In the next section we are going to describe some of the most important anatomical and physiological features of a mouse. The most important mouse biological and breeding data are summarized in Table 1.

Teeth and osseous system

The dental formula is 1/1 incisors, 0/0 canines, 0/0 premolars, 3/3 molars. The incisors in both jaws are bow-shaped and are continuously erupting and will overgrow if malocclusions occur.

Table 1. Some of the most important mouse biological data (Harkness and Wagner 1995; Poole et al. 1999)

Adult male body weight	20-40 g (strain dependent)
Adult female body weight	20-40 g (strain dependent)
Sexual maturation in male	6 weeks
Sexual maturation in female	5 – 8 weeks
Breeding onset for male	50 days
Breeding onset for female	50 – 60 days
Estrous cycle duration	4 – 5 days
Estrus duration	12 h
Gestation Period	18-21 days
Litter size	2-12 (average 6-8)
Weaning age	18 – 28 days
Life Span	1.5-3 years
Body Temperature	36-38 °C
Food consumption	15 g/100 g BW/day
Water consumption	15 ml/100 g BW/day
Heart rate	500-850 beats/min
Respiratory Rate	60-220 per minute

The normal vertebral formula is C7 T13 L6 S4 C28. The mouse normally has 13 pairs of ribs, the cranial 7 pairs are true ribs which articulates with the sternum. The other 6 pairs are false ribs and three of them which are the most cranial, connect to the caudal-most true rib. The others are floating and are not attached to any other osseous structures. Front and rear foot both have five digits each.

Respiratory and gastrointestinal system

The left lung has one, the right four lobes (superior, middle, postcaval and inferior). The alimentary tract is similar to other mammals and consists of the oesophagus, stomach, small intestine which is divided into duodenum, jejunum, ileum and the large intestine with caecum, colon and rectum. The stomach is divided into cardiac and pyloric parts, the left is nonglandular cardiac part and the right is glandular pyloric section. The liver consists of four main lobes: a large median, a right and left lateral and a left caudal lobe. A gall bladder is attached to the caudal surface of the median lobe. The pancreas is composed of many irregular shaped lobes of varying size, distributed in the mesentery of the duodenum with its distal end in close proximity with the spleen (*Roscoe B. Jackson Memorial Laboratory and Green 1966*). It is compounded of exocrine and endocrine part, the latter contains groups of cells, called islets of Langerhans. They consist of a heterogeneous population of cells (Elayat et al. 1995): insulin-releasing beta cells (65–90%) that form the core of the islet, glucagon-releasing alpha-cells (15–20%), somatostatin-producing delta-cells (3–10%) and pancreatic polypeptide-producing PP-cells (1%) are usually located on the surface. In newborn mice islets are mainly aligned along the ducts while the exocrine tissue is peripheral (Meneghel-Rozzo et al. 2004). Mice lack the appendix.

Urogenital system

The right kidney is normally anterior to the left kidney. The urine is clear, yellow and quite concentrated with a large amount of protein that are normally excreted in the urine of mice. The pH of normal mouse urine is about 5.0.

In males the inguinal canal remains open, and the testes may be retracted into the abdominal cavity. Males typically have an *os penis*, a small bone over the urethra near the tip of the penis. Preputial glands are paired structures and lie subcutaneously near the tip of the prepuce. In females the reproductive tract includes two uterine horns that combine to form the median corpus. The clitoral glands lie subcutaneously just lateral to the opening of the urethra. Since the placenta of the mouse is *hemochorial*, maternal immunoglobulins are transferred to the pups across the placenta. They are also transfered across the intestinal epithelium from colostrum for 16 days after parturition (*Roscoe B. Jackson Memorial Laboratory and Green 1966*). The mouse female has five pairs of nipples, three pairs over the ventral thorax and two on the abdomen. Males can be distinguished from females by the scrotal sac containing the testes and by a longer anogenital distance.

Gland associated with the eye

The extraorbital lacrimal gland is situated slightly below and in front of the ears. It is composed of small lobes and produces a secretion that lubricates the globe.

The intraorbital lacrimal gland is found near the lateral corner of the eye and as the extraorbital gland, produces a secretion that lubricates the globe.

The harderian gland is horseshoe shaped, lies behind the eyeball and partially encircles the optic nerve. It produces a secretion that lubricates the eyelids (Suckow et al. 2001).

Body weight, body temperature and senses

The small size and resulting large surface area/ body weight ratio makes mouse susceptible to changes in environmental conditions. The normal body temperature is 36 – 38 °C and can be easily affected by small changes in environmental temperature. This may have consequences in modified physiological responses of the animal. Mice alter heat loss by altering blood flow through tail. The acute hearing of mice makes them highly sensitive to ultrasounds and high pitched noises inducing a stress response which may result in cannibalism of pups by the female mouse. Mice can detect sound from 10 Hz up to 100 kHz, but are the most sensitive for sounds in a range from 15 kHz to 20 kHz and around 50 kHz (Jennings et al. 1998). They use ultrasonic calls in social interactions, pup distress calls, alarm calls and male mating calls. The well developed sense of smell is used to detect pheromones used in social interactions described in next section. Smell is the most important mouse sense, crucial for avoiding predators, feeding mating, parenteral, territorial and social behaviours. Comparing to the highly developed hearing and smelling, mouse vision is poor. They are adapted to life in low levels of light and thereby vision is less critical sense for normal behaviour. Mice are unable to detect red colour, which is useful for observation purposes. On the other hand strong white light can cause retinal atrophy and should therefore be avoided (Jennings et al. 1998).

Mice have fixed focus with very deep filed of focus. Their vision is sensitive to movement, not form. Mice lack red cones, but have UV cones.

Puberty and pairing

Female mice reach puberty at 5-8 weeks and males at six weeks of age. Females can conceive when they are as young as 23 days old and mice breed that early usually produce small litters (Laboratory 2009). Generally laboratory mice can breed for about 7-8 months, producing 4 or more litters. Reproductive life span of different strains varies, some of them can produce only one or two litters, usually because strain-specific characteristics affect their fertility. Mice are polyestrous and breed all year round. The ovulation is spontaneous. The duration of the estrous cycle is 4-5 days and it is divided into 5 stages, proestrus, estrus, metestrus 1, metestrus 2 and diestrus. Estrus lasts for about 12 hours and like mating and ovulation tend to occur during the dark phase of light cycle (Roscoe B. Jackson Memorial Laboratory and Green, 1966).

Group-housed females introduced to a male or to his odour, will result in synchronization of their estrous cycle in approximately 72 h, this phenomenon is known as Whitten effect. Prolonged absence of male pheromones results in a state of anestrus, but 40-50% of the females will resume estrus and cycling when they are exposed to a male or his odour. Another contrast is the Bruce effect, where a pregnant female aborts after being exposed to a male or his odour within four days of breeding (Suckow et al. 2001).

Laboratory mice breed best when the temperature is between 20-26 °C, the humidity 40-60 % and because mice generally breed at night a consistent and uninterrupted light/dark cycle should be maintained. Mating results in the formation of a vaginal plug which can be detected to confirm mating. Vaginal plug is a whitish mass composed of coagulated secretion from coagulating and vesicular glands of the male mouse and it is typically present for 8-24 h following breeding. If the plug is not present, the female should be left in a cage with a male for one more day or until the plug is found. The first day of gestation is considered to be the day after the plug is found (Roscoe B. Jackson Memorial Laboratory and Green, 1966).

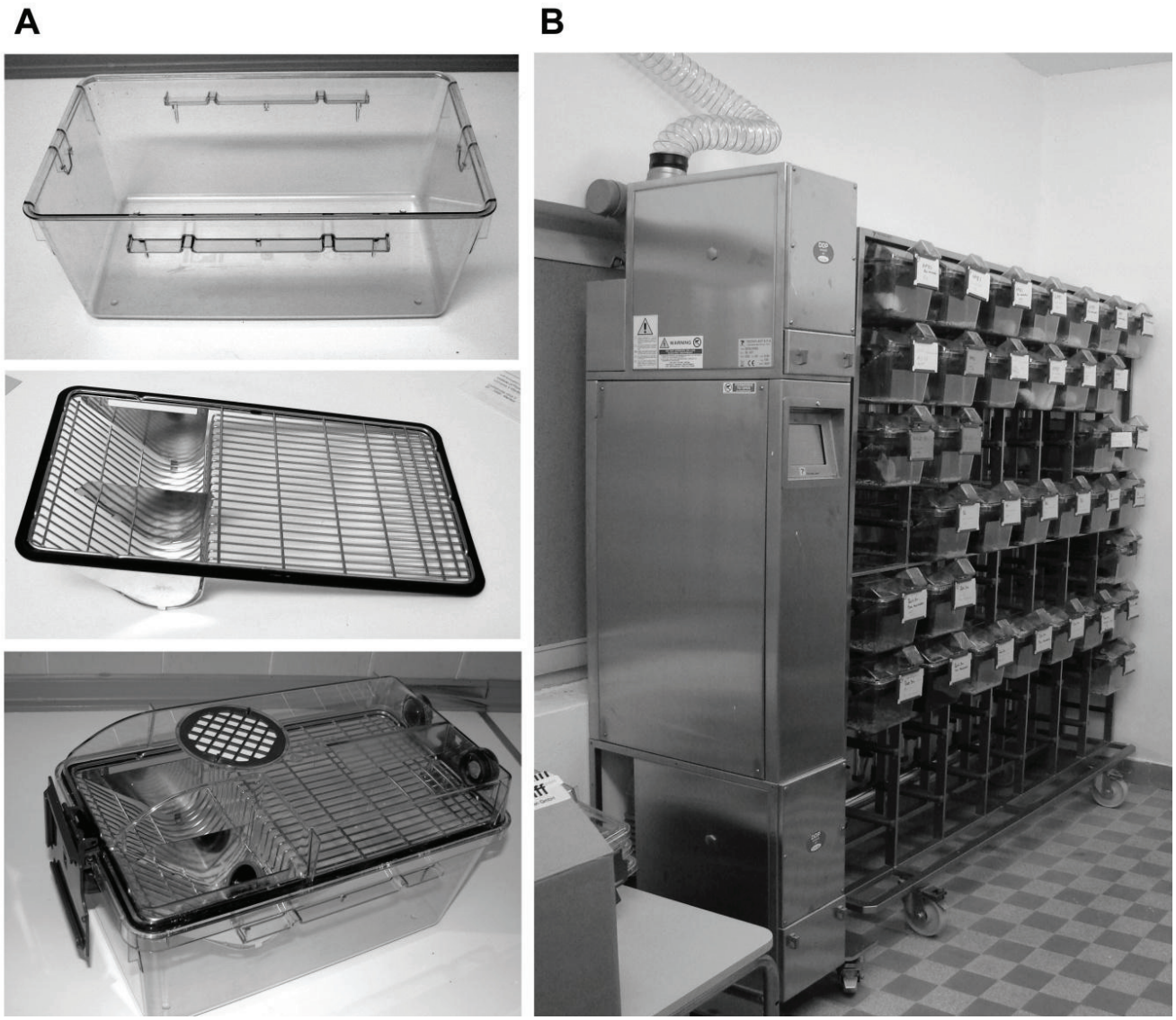

Figure 1: A - Polycarbonate cage (upper panel) with wire bar lid (middle panel) and plastic lid to isolate cages (lower panel) B - Ventilated rack with cages.

The gestation period lasts 18 and 21 days and 2-12 pups may be born, depending on the strain. Mice are weaned at 3 weeks by which time have fur and their eyes and ears are open.

Behaviour and housing

Mice are nocturnal, burrowing and climbing animals with a peak of activity during the dark period (Kaliste 2004). They are active, highly explorative and social animals with a wide range of social organizations from strong territoriality by reproductively-acting males to large colonies with well developed social hierarchies. The dominant male defends his territory. Since mice are social animals it is recommendable to keep them in groups rather than in individual cages. After weaning mice are separated by sex and housed together in small groups with no more than 5 animals per cage. Females of any age can be housed together. To minimize fighting, weaned males can only be housed with their littermates and only until they are exposed to females. Males from different litters can be grouped together only if they are less than 4-5 days apart in age. Males that have been used for breeders must always be housed singly otherwise they will kill each other. Infanticide is normal part of mouse reproductive behaviour. Normally males never meet pups that are not theirs and kill other males' pups and favour their own. Both copulation and postcopulatory cohabitation with pregnant females reduce infanticide and enhance paternal responsiveness in male mice (Elwood 1986). Females are infanticidal unless when lactating. They are less likely to kill pups from familiar females.

Although this is not natural, particular male mice will form stable hierarchies (Kaliste 2004).

Laboratory mice are usually bred and housed in cages made of polycarbonate or polypropylene with solid floors and walls. Lid is usually from wire bar and used to hold the water bottle and feed (Fig. 1A). The system with ventilated racks which supply filtered air is the most appropriate way for mice husbandry. It is applicable because enables the husbandry of many cages at a relatively small area (Fig. 1B). Furthermore, ventilation can be regulated, air exchange can be manually set and a positive or negative pressure can be applied. Since mice are nesting animals they prefer sawdust strewing for digging, nesting and resting rather than grid floor. Bedding from corncob is less comfortable for resting and nesting but it better absorbs urine and therefore reduces bad odour. Nesting material, such as paper towels, can provide the shade and give the opportunity to regulate microclimate. Generally, bedding material should be non toxic or harmful for animal, absorbent, but not dehydrating for neonates, not excessively dusty and of course economical. Sometimes mice are housed on wire mesh bottom cages to allow collection of *feaces* and urine or to prevent contact with bedding. This type of housing is not preferred and may be used only when it is necessary for experimental design.

The minimum cage size for maintaining laboratory animals depends on the strain, group size, age of the animals, their familiarity with each other and their reproductive conditions. The floor area for a single mouse should be greater than 180 cm^2 (it is weight dependent) and for a female with a litter more than 200 cm^2 (Ministry of Agriculture 2006). The European Union is considering about a directive on laboratory mouse care that would mandate specific rodents' enrichment but the consensus for what constitutes good animal practice has remained elusive. It is unclear whether such variability in cage enrichment affects scientific data but researchers generally agree that for most types of experiments basic enrichment will not change the data. Cage enrichment can be accomplished by adding nesting material such as shredded paper to the mouse cage, a PVC tube or paper tubes for mice to hide. Furthermore, housing mice in group rather than singly can contribute to enrichment. Nowadays, many enrichment products are commercially available but we have to be aware that most of these products are not actually of use to animals, they are not always well tested and would not benefit the species they are geared towards. However, basic enrichment may have a positive effect by to some extent creates more natural environment (Katsnelson 2009). It is important for providing stimuli or substrates for normal behaviour, increases the range of normal behaviour and decreases abnormal behaviour. If mice are able to biuld nest properly, nests are insulating and keep mice warm.

Feeding

Mice are omnivorous animals and are typically fed *ad libitum* unless food restriction is required as a part of the research protocol. Feeding behaviour in mice shows a diurnal pattern with a majority of food consumed during the dark period. Food intake is approximately 15 g/100 g body weight/day (BW/day) and is generally offered as hard pellets. The hardness of pellets is important for sharpening the teeth. If the food is too soft it will crumble easily and more food will be needed. If the food is too hard mice are not able to chew it. The food should be as fresh as possible and should be stored at cool, dry place, separated from the breeding room. Water should be provided from bottles or automatic watering system. Water bottles must always be cleaned properly and the water should be as fresh as possible or changed at least once a week. Water intake is approximately 15 ml/100 g BW/day.

Pelleted natural ingredient diets are used to feed all rodents and are composed primarily of cereal grains which are supplemented with additional protein, vitamins and minerals. Due to the nature of this type of diet the exact composition can vary substantially from lot to lot. The water provided to animals is chlorinated tap water.

Breeding animals have different nutritional requirements, which if not provided, will have a harmful effect on reproductive performance. Light cycles are important in breeding mice and are provided with 12 hours light/dark cycle. Deviations from this cycle will influence the reproductive performance.

MICE HANDLING

When handling mice it is advisable to wear gloves to prevent the development of allergies due to direct contact with animal allergens. Mice are usually caught and lifted by the tail with the thumb and forefinger or by the use of smooth-tipped

forceps. With this method mice can be transferred to another cage, identified, examined or sex may be determined. When handling with pregnant mice or very obese mice, their feet should be supported by the second hand. For more effective control, the mouse may be held by the tail and placed on a table or other surface, preferably to a meshed cage lid that the mouse can grasp to. Besides, the loose skin over the neck and shoulders need to be grasped with thumb and fingers. This manoeuvre should be performed promptly not to take the risk of being bitten. Once the mouse is grasped correctly, the head is adequately controlled. When finishing examination, mice should not be dropped into the cage because this may result in spinal fracture, but should be lowered into the cage and released upon contact with the bedding.

Mice less than two weeks of age can be handled by grasping the loose skin over the neck and shoulder with thumb and forefinger or smooth tipped forceps. Neonatal mice should not be handled during the first few days after birth to avoid cannibalism or litter abandonment. When handling newborn mice, it is important to transfer the mother first, to prevent aggression from her. The neonates may be handled using plastic gloves to avoid contamination with human scent.

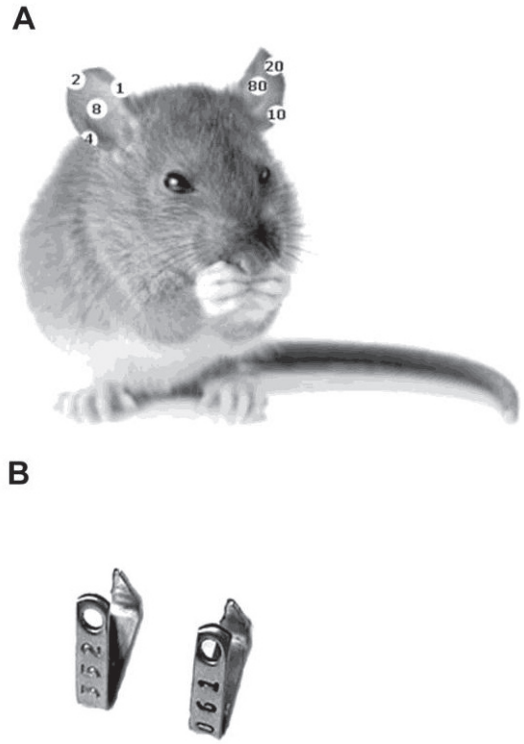

Figure 2: Identify a mouse with two most frequently used methods.

A - Mouse identification with ear punching. Right ear is punched for numbers from 1 to 10 and both ears are punched if we need to identify mouse with large numbers.

B - Metal ear tags for small animal identification.

IDENTIFICATION

To uniquely identify pups different methods can be used. Ear punching is simple, inexpensive but hard to read but sometimes ambiguous and numbers are limited. Numbers from 1-10 can be obtained by combining the numbers. For numbers greater than 10, both ears have to be punched (Fig. 2A). Metal ear tags (Fig. 2B) are relatively inexpensive and numbering is unique but they are hard to read if tags are not distinctly marked and there is a great chance of loosing tags. Other not often used methods are toe clipping which is less humane and therefore not recommended, tattooing which is difficult and may fade with time, and microchips which are permanent and safe but expensive, difficult to apply and requires expensive reader. Tail clipping is often used in genetically modified mice in order to take tissue samples from offspring for identifying their genetic makeup.

HEALTH MONITORING

Good health is not only crucial for the animal's well being but also for the quality of the data obtained from experiments. Therefore the health status of the animals should be defined. Husbandry itself should aim to maintain animals in the highest standards of health, to minimize clinical and subclinical diseases and to prevent zoonotic risks for humans. Therefore every breeding laboratory should have individual monitoring programs to assure proper health monitoring, protocols to define the microbiological status of the animals in order to take into consideration the presence or absence of certain microorganisms carried by the animal. Since stereotypic behaviour may be sign of inadequate environment, refinement of husbandry may help to minimize stress and the susceptibility for diseases. When dealing with clinical diseases a proper veterinary care should be provided and in case of the sudden death or stillborn pups, the pathomorphological examination has to be assured to determine the cause of death and to prevent the transmission of the disease.

REDUCING THE NUMBER OF ANIMALS USED IN DIABETES MELLITUS RESEARCH

Due to Western life style diabetes mellitus has globally become one of the most important diseases of the modern society. Despite the current way of treatment it has become a major cause of death. Insulin plays a central role in controlling body's metabolism and the lack of insulin secretion leads to the serious metabolic disturbances. Because of the vast economic importance of the disease, insulin release has become intensively studied and several novel approaches have been applied.

Reduction of the number of animals used in biomedical research is the primary goal. This can be accomplished by using cell lines instead of living animals. Pancreatic beta cell lines have been used for many years in diabetes mellitus research. Immortal cells lines were established by using X-rays or viruses to induce insulinomas, *in vitro* transformation and derivation of cells from transgenic mice or even non-islet cells (Ulrich et al. 2002). Only a few of

these attempts have been successful. Some cell lives are poorly responsive to glucose, others respond to glucose well but their concentration-dependence curve is markedly shifted to higher sensitivity (Efrat 2004). Next, isolated pancreatic beta cells may be used to study the physiological function of endocrine pancreas and the mechanism of insulin secretion. Standard approaches in physiological experiment included patch-clamp recordings on isolated and dispersed beta cells and isolated islets of Langerhans. A few years ago a new preparation has been developed (Speier and Rupnik 2003) where the relationship between the different anatomical structures is preserved and the gross morphology of the pancreas is not disrupted (Fig. 3). Freshly prepared pancreatic slices are a novel *in situ* preparation for electrophysiological and imaging studies on beta cells and other cell types in the pancreas. By this unique approach beta cells are not disturbed enzymatically or physically and the islet capsule remains intact which prove near-physiological conditions. Another benefit of this technique is the fact that we need only one animal for the tissue slice preparation while the experiments on isolated cells require 2 or 3 animals.

The major advantage of pancreas slices technique has been its application in studying prenatal and perinatal morphology and development of endocrine and exocrine pancreas (Meneghel-Rozzo et al. 2004), the determination of physiological regulation of ATP dependent K^+ channels (KATP) which inhibition seems to play a pivotal role in signal transduction of glucose-induced insulin release (Speier et al. 2005). During the past few years many physiological properties of pancreatic endocrine cells were described through the use of pancreas tissues slices. We described the physiological role of electrical coupling in pancreatic beta cells (Speier et al. 2007), the compensatory changes found in diabetic Goto Kakizaki (GK) rats, the role of the lack of peripheral serotonin (Paulmann et al. 2009) and several other physiological disturbances in gene ablated animals.

CONCLUSIONS

Laboratory animals have been the experimental model of choice in research for many years and many important discoveries were established through the use of animals. On the other hand we have to be aware of animals' suffering when using live animals for the experiments and because of that reason we have to strive for reducing the number of animals in researches. Experiments with live animals should be limited to the fields where we have no alternatives and more effort should be directed into developing experimental methods and approaches that include only organs and tissues taken at the animal's time of death. Preparations such as pancreatic slices are an excellent illustration proving that experiments can be performed in near-physiological conditions without requiring the live animal for the research.

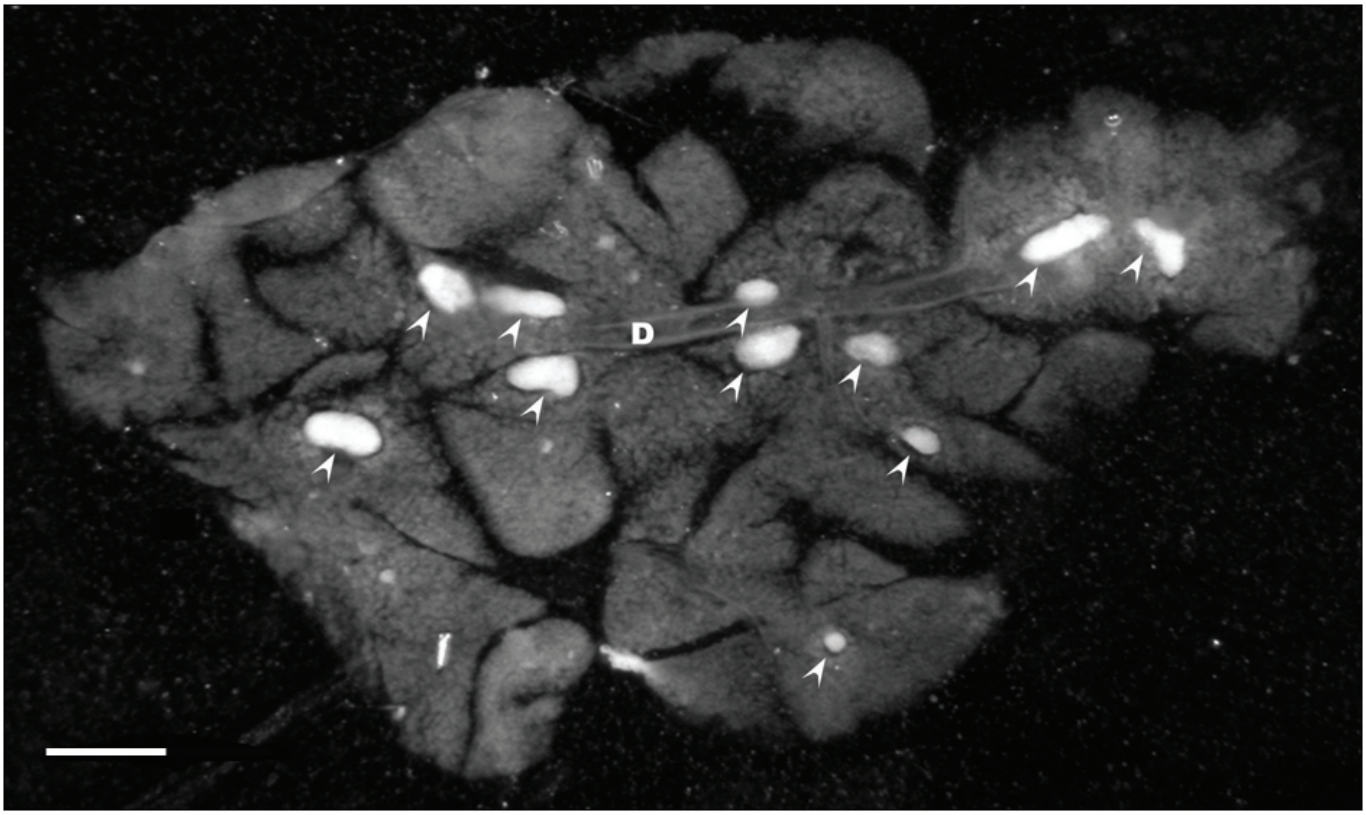

Figure 3: Stereomicrograph of the pancreatic slice. The bulk of the tissue consists of exocrine cells. The islets of Langerhans are seen as bright white structures indicated with arrow heads. The pancreatic duct is indicated with D. Scale bar: 1 mm.

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