Doctoral Programme: Biological responses to environmental challenges (BIOREC) - carry over across life phases and generations

KEY WORDS: Environmental Stress; Vertebrates; Endocrinology; Reproductive and Developmental Physiology; Ecological and Environmental Physiology; Phenotypic Plasticity; Epigenetic Effects

Worldwide, there is concern about interactions of man-made environmental stress along hormonally regulated pathways in animals and humans - during their early life stages in particular.

In addition, there is increasing evidence that (a) even the very early environment experienced by the embryo can affect developmental outcomes, (b) such impacts of environmental challenges may persists over life phases and generations, and in the end, (c) may negatively affect the fitness of the organisms.

> **"ENVIRONMENTAL CHALLENGES AND STRESS RESPONSES":** In all biota, phenotypic manifestations are modulated by genotype-environment interactions. In fact, changes in all natural or anthropogenic, biotic or abiotic environmental factors may cause a series of stress responses in organisms. In response to changing environments, organisms may modify their physiology and morphology, development and growth, or behaviour, within their genetically determined limits of regulatory capacity. "**ENDOCRINOLOGICAL STRESS RESPONSES":** Hormones play a central role in communicating information in organisms and thus initiating and coordinating physiological responses to environmental challenges. In vertebrates, particularly unpredictable environmental challenges (Labile Perturbation Factors, LPFs) activate the hypothalamo-pituitary-adrenal hormonal axis, the so called „stress-axis" (Henry and Stephens „Coping-Predictability Concept"). Glucocorticoids and catecholamines are central to the front-line defence for vertebrates under stress conditions. "**FITNESS RELATED STRESS RESPONSES":** Environmental challenges negatively affecting the fitness of organisms translate into impairment of reproductive success eventually. "**CARRY OVER OF STRESS RESPONSES":** Stress responses resulting from early life stage exposure to environmental challenges may persist through life phases and generations. Fitness related parameters comprise direct measures of reproductive performance but also measures of development, growth and survival, behaviour and challenge performance.

Research in the BIOREC Doctoral Programme collectively aims at understanding the role hormones play in regulating the effect cascades which translate environmental changes into fitness related changes through phenotypic variability in vertebrates. We are particularly interested in the stress response as indicated by "Endocrinological and fitness related stress profiles" in a set of in vitro and in vivo vertebrate models relevant in reproductive and developmental biomedical research.

In these studies a variety of techniques (Biochemistry, Histology and Histochemistry, in vitro cell, tissue and embryo culture, and biometry) will be employed. We will study shorter term stress responses particularly in domestic livestock mammals (horses, cows, sheep) and birds (chick), and longer term responses particularly in guinea pigs, amphibians and fish.

Within this framework, BIOREC all together aims at answering the questions: (a) What is the spontaneous and induced intra- and interspecific variation in the selected stress response parameters, and (b) what are the relationships between the different categories of stress responses over life phases and generations?

as indicated by the following major stress response categories (Project Cluster) analysed in the BIOREC research design:

(1) "Endocrinological stress response profiles" comprising a set of

i. hormonal parameters (several hormones and metabolites; steroid hormones and catecholamines in particular), and

ii. histological and histochemical parameters (hormonal and reproductive system in particular).

- Endocrinological effects of stress will be studied in all BIOREC PhD Projects also during the initial programme phase.
- Individual PhD projects supervised in this cluster will focus on the chemical physiology of "shorter term" stress responses and are supervised by the VUW Units "Biochemistry", "Histology and Embryology".

(2) "Fitness related response profiles" comprising a set of indicators for

i. Reproductive and developmental performance (survival, development and growth spanning gametic to endotrophic developmental phases),

- Reproductive effects will be studied in all BIOREC PhD Projects although at different degrees of depth during the initial project phase.
- Individual PhD projects supervised in this cluster will focus on the reproductive and developmental physiology of "shorter term observation" (partial life phase to multi-life phase)

stress responses and are supervised by the *VUW Units "Artificial Insemination and Embryo Transfer " and "Animal Breeding and Genetics".*

ii. Reproductive and developmental performance (as indicated by survival, development and growth spanning gametic , endo- and exotrophic developmental phases), and behavioural performance indicators (locomotor activity and challenge response).

- Developmental and behavioural effects will be studied in all BIOREC PhD Projects although at different degrees of depth during the initial project phase.
- Individual PhD projects supervised in this cluster will focus on the ecological and environmental physiology of "longer term observation" (multi-life phase to multi-life cycle) stress responses and are supervised by the *VUW Units "Animal Husbandry and Animal Welfare", "Aquatic Ecotoxicology", and " Wildlife and Ecology".*

The **BIOREC DOCTORAL PROGRAMME** involves a multidisciplinary consortium of seven VUW RESEARCH UNITS ***("Biochemistry", "Histology and Embryology", "Artificial Insemination and Embryo Transfer " and "Animal Breeding and Genetics", "Animal Husbandry and Animal Welfare", "Aquatic Ecotoxicology", and "Wildlife and Ecology")*** and has been designed following the Austrian Science Fund (FWF) format as to be of a decade long continuity. It therefore **involves nine INDIVIDUAL RESEARCH PROGRAMMES** aiming at continuing progress in networking and focus. Each INDIVIDUAL RESEARCH PROGRAMMES will comprise a **series of PhD PROJECTS** out of which the first are open for application now. Currently, the ASSOCIATED PARTNERS of BIOREC are the VUW "***Clinic for Ruminants" and the "Clinic for Poultry, Petbirds, Reptiles, Fishes"***.

The following four BIOREC PhD Projects are currently open for applications and planned to start in October 2007:

BIOREC PhD Project 1.1: Adrenocortical activity during different life stages in ruminants.

BIOREC PhD Project 3.1: Determination of the expression of glucocorticoid receptors and cortisol-metabolizing enzymes in the testes of horses of different reproductive stages and breeds.

BIOREC PhD Project 4.1: Interaction of early preimplantation embryos and disturbances in the cultural environment.

BIOREC PhD Project 6.1: Endocrine and developmental effects of early life stage exposure to endocrine modulating reference compounds in zebrafish (*Danio rerio*) and African clawed frog (*Xenopus laevis*).

The following BIOREC PhD Projects are planned to be opened for applications in 2008 and start in October 2008:

BIOREC PhD Project 2.1: Uterine glands in vitro: establishment of a three-dimensional endometrial cell culture system and evaluation of steroid hormone mediated functions.

BIOREC PhD Project 5.1: Effects of maternal deprivation on welfare, behaviour and challenge responses of dairy calves.

BIOREC PhD Project 7.1: Reproductive effects of cadmium on European brown hares

BIOREC PhD Project 8.1: Effects of early life factors on stress response of guinea pigs.

BIOREC PhD Project 9.1: Maternal communication of environmental challenges to offspring in chicken

The following four BIOREC PhD Projects are currently open for applications and planned to start in October 2007:

INDIVIDUAL BIOREC RESEARCH PROGRAMME RP-1: INFLUENCE OF LIFE STAGE, SEX AND PLASMA BINDING GLOBULINS ON ADRENOCORTICAL ACTIVITY IN DOMESTIC LIVESTOCK (RUPERT PALME, ERICH MOESTL: *"BIOCHEMISTRY"*)

BIOREC PhD Project 1.1: Adrenocortical activity during different life stages in ruminants.

In animals, glucocorticoids (GC) are front-line hormones to overcome stressfull situations. Although their quantification in blood samples is possible, sampling itself is stressful and thus may confound the results. Therefore non-invasive monitoring of adrenocortical activity by means of fecal GC metabolites offers several advantages and has been successfully developed and applied to various species of mammals and birds by our group. Still, profound knowledge about adrenocortical activity and amounts of different GC during life stages of an animal and about the effects of plasma GC transport on steroid metabolism and excretion is still lacking. Those questions will be addressed in ruminants (cattle and sheep) in order to provide a sound background for a broad application of these non-invasive methods in the future.

INDIVIDUAL BIOREC RESEARCH PROGRAMME RP-3: RESPONSE OF REPRODUCTIVE FUNCTION (SPERMATOGENESIS, STEROIDOGENESIS) TO ENVIRONMENTAL CHALLENGES IN THE STALLION (CHRISTINE AURICH: *"ARTIFICIAL INSEMINATION AND EMBRYO TRANSFER"*)

BIOREC PhD Project 3.1: Determination of the expression of glucocorticoid receptors and cortisol-metabolizing enzymes in the testes of horses of different reproductive stages and breeds.

Species that are naturally frequently exposed to stress (e.g. equids in their natural habitat) have to be able to reproduce despite a frequent fluctuation of glucocorticoid concentrations. This suggests a low responsiveness of their gonads to cortisol. Due to domestication and selection, the gonadal responsiveness might change and increase. Under natural conditions, stallions strictly live in social groups with mares or other stallions. We will test the hypothesis that stress caused by isolated housing of domesticated stallions under the conditions of the modern horse breeding industry may contribute to low fertility. The endocrine response to a controlled stress situation in adult stallions will be investigated and the influence of glucocorticoids on reproductive testicular functions will be characterized. Mechanisms will be compared between stallions of a wild-type and a domesticated breed to investigate possible phaenotypic changes caused by domestication. The study will help to investigate an involvement of stress in the pathogenesis of sub- and infertility in stallions kept under the conditions of the modern horse breeding industry.

INDIVIDUAL BIOREC RESEARCH PROGRAMME RP-4: RESPONSES IN FEMALE REPRODUCTIVE FUNCTION TO ENVIRONMENTAL CHALLENGES (URBAN BESENFELDER: *"ANIMAL BREEDING AND GENETICS"*)

BIOREC PhD Project 4.1: Interaction of early preimplantation embryos and disturbances in the cultural environment.

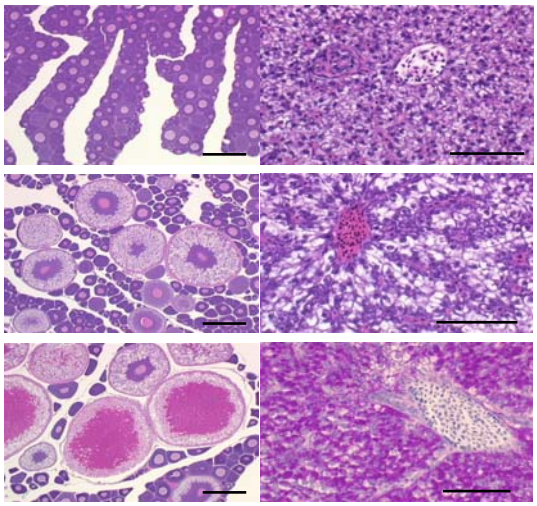
There is an urgent need for a concerted and sustained investment in agricultural research to solve problems in bovine fertility, caused by multiple reasons such as high milk yields, health problems and management systems. Follicular dynamics followed by embryo development represent an unique indicator for the assessment of biological responses to environmental challenges. During the past decades, basic and experimental studies on in vitro production (IVP) of bovine embryos have generated structural and functional information on oocyte development and quality, fertilization and conceptual development. This information has provided new insight, not only into this in vitro technology per se but also into the factors contributing to fertility in cattle. Despite tremendous progress, causal explanation of fertility problems in cows is still insufficient, and therefore, the use of in vitro-derived bovine embryos is still significantly lower than that of embryos, which were completely developed in vivo. It is well known, that in vitro culture conditions can partially simulate the requirements for embryo development. But, artificial culture conditions may also adversely affect embryonic and fetal growth, leading to severe reproductive disorders. Therefore, it still remains open as to which extent in vitro systems provide predictive information for application in animals in vivo. The current project aims at comparing the viability of oocytes and embryos temporally cultured in vivo and in vitro, and understanding the hormonally mediated processes underlying fertility problems in cows by monitoring steroid hormone presence and availability.

INDIVIDUAL BIOREC RESEARCH PROGRAMME RP-6: ENDOCRINE EFFECTS OF ENVIRONMENTAL POLLUTION ON AMPHIBIAN AND FISH LIFE HISTORY AND PERFORMANCE (BRITTA GRILLITSCH: *"AQUATIC ECOTOXICOLOGY")*

BIOREC PhD Project 6.1: Endocrine and developmental effects of early life stage exposure to endocrine modulating reference chemicals in zebrafish (*Danio rerio*) and African clawed frog (*Xenopus laevis*).

Environmental pollution forms an important category of anthropogenic threats to wildlife and humans. Endocrine Disrupting Chemicals (EDCs) are of special concern in that they are exogenous substances that have the potential to alter functions of the endocrine system and consequently cause adverse health effects. Reproductive and developmental effects resulting from early life phase exposure to EDCs are of priority research interest particularly so for longer term and multi-stressor mediated effects. However, other than estrogenic effects resulting from single-compound exposure have rarely been analysed to date. In this project, we will focus on (a) the aquatic exposure pathway, (b) freshwater vertebrate model species (amphibians and fish), (c) embryonal and larval exposure, (d) interactions along the hypothalamo-pituitary-adrenal and -gonadal axes, and (e) both, shorter and longer term effects as indicated by changes in life history and performance traits. In order to reliably assess exposure-response relationships, experiments will be conducted under highly defined and controlled conditions and employing a set of model test compounds according to state of the art principles on quality assurance and test designs (e.g., OECD, EC TGDs). The applicability of non-invasive monitoring techniques will be validated. To assess the extrapolative power of results derived from laboratory model systems established in Ecotoxicological Risk Assessment, we will analyse the intra- and interspecific variability in a multi-level set of structural and functional indication criteria for single- and multi-stressor exposure, and shorter and longer term observation scenarios.

The following BIOREC PhD Projects are planned to be opened for applications in 2008 and start in October 2008:



INDIVIDUAL BIOREC RESEARCH PROGRAMME RP-2: REPRODUCTIVE EFFECTS OF ENVIRONMENTAL CHALLENGES ON CELL AND TISSUE STRUCTURE AND FUNCTION IN VIVO AND IN VITRO (INGRID WALTER: *"HISTOLOGY AND EMBRYOLOGY"*)

BIOREC PhD Project 2.1: Uterine glands in vitro: establishment of a three-dimensional endometrial cell culture system and evaluation of steroid hormone mediated functions.

Several aspects of function and dysfunction in reproductive organs have to be studied in experimental approaches. However, tests on living animals - though important in veterinary medicine - are limited due to ethical reasons and high costs, especially with domesticated animals such as dogs, cows, and horses. Moreover, effect mechanisms and effects of single factors to specific cells or tissues often can only be studied in cell culture systems. Three-dimensional cell and tissue culture systems with fully differentiated cells are suitable to mimic complex in vivo situations but have rarely been established for veterinary applications to date. Although cell culture models cannot fully replace clinical studies or studies on living animals, they give valuable information and therefore, the expenditure of establishing three-dimensional in vitro models is strongly justified. This research aims to establish three-dimensional cell and tissue culture systems with fully differentiated cells focusing on female reproductive organs, endometria in particular, and examine their applicability for studying (a) endometrial in vitro fertilization support (feeder layers), (b) the molecular basis of normal reproduction, (c) mechanisms of disorders in reproductive organs, and (d) screening assays of substances that influence reproduction (endocrine disruptors).

INDIVIDUAL BIOREC RESEARCH PROGRAMME RP-5: EFFECT OF THE EARLY SOCIAL ENVIRONMENT ON WELFARE AND LATER CHALLENGE RESPONSES IN DAIRY CATTLE (SUSANNE WAIBLINGER: *"ANIMAL HUSBANDRY AND ANIMAL WELFARE")*

BIOREC PhD Project 5.1: Effects of maternal deprivation on welfare, behaviour and challenge responses of dairy calves.

Effects of different social experiences during early life phases on later personality characteristics influencing social skills and coping success in social and non-social challenging situations have been shown in several species. However, large mammals with a long juvenile period such as cattle have rarely been investigated, although the permanence of possible effects during the first weeks of life up to adulthood is of special interest. In dairy cows, early social deprivation is quite common, while development of appropriate social skills may be essential for coping successfully with the later social challenges in a dairy herd and thus for welfare state, disease susceptibility and (re)production of dairy cows. The aim of the project is to investigate potential effects of the early social environment on welfare and health of dairy cattle from birth up to their first lactations and underlying mechanisms. The specific objectives are to investigate the potential effects of early social environment (1) immediately on welfare (health, stress physiology, behaviour), behaviour and challenge responses of dairy calves and (2) in the long-term on later ability to cope with social and non-social environmental challenges such as regrouping, reactions to novelty or humans. Calves running with and suckling their mothers until weaning at the age of three months will be compared with artificially reared calves kept in groups from the second week of life and will be followed until finishing their first lactation. Non-invasive physiological (heart rate variability, faecal cortisol metabolites) and behavioural parameters will be used in the home environment and undisturbed situation as well as in test situations and health and production parameters recorded regularly.

INDIVIDUAL RESEARCH BIOREC PROGRAMME RP-7: ENDOCRINE EFFECTS OF METAL POLLUTION ON FITNESS COMPONENTS IN MAMMALS AND BIRDS (FRIEDA TATARUCH: *"WILDLIFE AND ECOLOGY"*)

BIOREC PhD Project 7.1: Reproductive effects of cadmium on European brown hares

Reproductive performance is crucial for wildflife populations. Among many substances showing adverse effects on reproduction cadmium might play a remarkable role. Effects of this ubiquitous trace element with a broad range of industrial use and various emission sources, on reproductive endocrinology have been proven for humans as well as for animals. However, there are only few data on cadmium effects in free-ranging mammals, although many species may be exposed to high Cd-levels in their habitats. Among these are European brown hares (Lepus Europaeus Pallas, 1778). Their population sizes have declined remarkably during the last decades in many parts of Europe.

They feed to a great extent on plants known for high Cd-levels, like herbs, cereal grains etc. Additionally, Cd-exposure of hares may be enhanced by use of Cd-rich fertilizers (phosphate fertilizers, sewage sludge) in crop cultivation areas, which represent the preferred habitats for hares. So, cadmium might be one of the factors responsible for the hares' decline.

We therefore plan to analyse the Cd-concentrations in inner organs of hares in different regions in Eastern Austria. The contamination will be correlated with fertility parameters of the female hares in these areas, like the number of placental scars which corresponds with the number of implanted embryos during the last reproduction period of the female hare.

In the case a significant correlation can be proven the influence of Cd on fertility will be tested experimentally on other species, e.g. on lagomorphs.

INDIVIDUAL BIOREC RESEARCH PROGRAMME RP-8: EARLY LIFE PHASE INFLUENCES ON REPRODUCTIVE AND CHALLENGE PERFORMANCE IN MAMMALS (SUSANNE HUBER; *"WILDLIFE AND ECOLOGY"*)

BIOREC PhD Project 8.1: Effects of early life factors on stress response of guinea pigs.

The investigation of the effects of early life factors on later life events is an emerging field of biological and medical research. There is accumulating evidence that environmental and social conditions experienced during foetal and neonatal life may influence early development with potential long-term consequences for a variety of parameters such as growth, reproduction, susceptibility to diseases, and survival. Recent human studies on birth season effects on reproductive output raised the assumption that the environmental conditions experienced during early life may have long-term consequences for later reproductive performance. The effects of early environmental conditions on later stress response have not been examined yet although several studies show that early social experience may explain individual differences of stress physiology. Aim of the project is to investigate whether environmental and social conditions during early development, including both pre- and postnatal environmental and maternal challenges, may exert long-term effects on i) later stress response, and ii) later reproduction in guinea pigs, and iii) whether there are any interactions between both putative effects, by examining effects of challenges of the HPG- or HPA-axis, respectively, during the pre- and postnatal period on later stress responsiveness and reproductive function, respectively. The guinea pig will be used as model species as, owing to similarities between the guinea pig and human ovarian cycle, it is a suitable model for an experimental examination of effects of early life factors on later reproductive capacity found in humans.

INDIVIDUAL BIOREC RESEARCH PROGRAMME RP-9: PRENATAL CHALLENGES IN BIRDS (SOPHIE RETTENBACHER, RUPERT PALME, ERICH MOESTL: "BIOCHEMISTRY")

BIOREC PhD Project 9.1: Maternal communication of environmental challenges to offspring in chicken

Maternal stress is communicated to the offspring via hormones and triggers structural and functional changes that persist for the lifespan. In birds, there is clear evidence that environmental conditions are communicated to the offspring via hormones to optimize reproductive success. There are several indications that in reaction to altered environmental situations, elevated androgen content in egg yolk is found, in order to enhance offspring fitness and survival. However, the underlying physiological mechanisms for these non-genetic effects haven´t yet been elucidated.
The aim of this study is to investigate if maternal stress alters offspring physiology and behaviour in birds, and to study possible mechanisms how stress is communicated from the bird mother to her progeny.The project has the following main thread (Investigations will be done step-wise in several experiments!): Adult laying hens will be stressed prior to/during egg-laying, eggs will be analysed for hormone content, hatchlings will be tested*. A subset of the chicks will be raised and long-term consequences for the individual as an adult animal on e.g. fertility will be assessed. Investigations will be performed by a doctoral student, under the supervision of the project leader and in cooperation with our research partners in the Netherlands.
*Immunological measurements comprise testing humoral as well as T-cell mediated immune responses with widely used, standardised methods (inoculation with SRBC and PHA skin testing). Offspring behaviour will be asessed via open field test, novel object test, tonic immobility food competition and aggression.