Available online at www.sciencedirect.com

SCIENCE DIRECT®

Toxicology and Applied Pharmacology 207 (2005) S375 – S380

Toxicology and Applied Pharmacology

www.elsevier.com/locate/ytaap

Review

Predicting the risk of developmental toxicity from in vitro assays☆

Horst Spielmann*

National Center for Documentation and Evaluation of Alternative Methods to Animal Experiments (ZEBET), Federal Institute for Risk Assessment (BfR), Diedersdorfer Weg 1, D-12277 Berlin, Germany

Received 15 July 2004; revised 12 January 2005; accepted 21 January 2005
Available online 24 June 2005

Abstract

Reproductive toxicity refers to the adverse effects of a substance on any aspect of the reproductive cycle, including the impairment of reproductive function, the induction of adverse effects in the embryo, such as growth retardation, malformations, and death. Due to the complexity of the mammalian reproductive cycle, it is impossible to model the whole cycle in a single in vitro system in order to detect chemical effects on mammalian reproduction. However, the cycle can be broken down in its biological components which may be studied individually or in combination. This approach has the advantage that the target tissue/organ of a developmental toxicant can be identified. In specific areas of developmental toxicity, a number of useful and promising in vitro models are already available. The individual tests may be used as building blocks of a tiered testing strategy. So far, research has focused on developing and validating tests covering only a few components of the reproductive cycle, in particular organogenesis of the embryo, reflecting important concerns for teratogenic chemicals. During the last three decades, a number of established models and promising new developments have emerged that will be discussed, e.g. culture of mammalian embryos and embryonic cells and tissues and the use of embryonic stem cells.

Keywords: In vitro toxicity; In vitro culture; Embryo culture; Developmental toxicity; Embryotoxicity; Teratology; Embryonic stem cells; Chemical toxicity

Contents

Introduction

Reproduction is a continuous cycle, but for the purposes of developmental toxicity testing, it may be divided into pregnancy including prenatal and postnatal developmental toxicity as well as the remainder of the cycle, which is

☆ Presented at the ICTX 2004, in Tampere Finland, July 11 – 15, 2004.

* Fax: +49 1888 8412 2958.
E-mail address: spielmann.zebet@bfr.bund.de.

0041-008X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2005.01.049

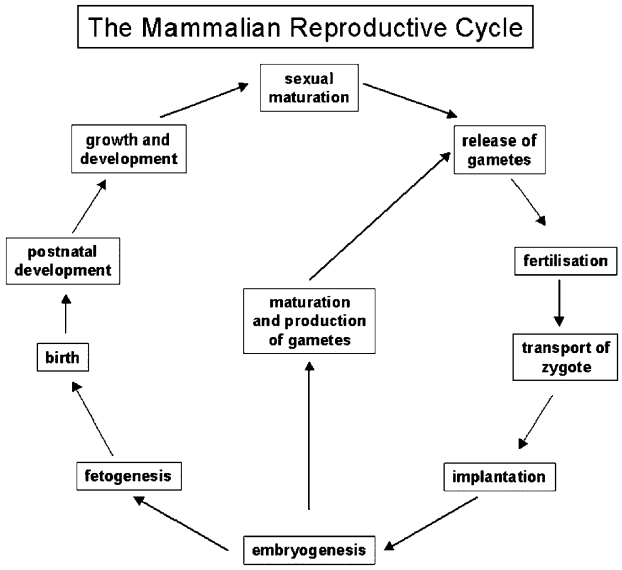

Fig. 1. The mammalian reproductive cycle. The essential steps of the continuous reproductive cycle of mammals are shown. It is covering developmental toxicity as well as fertility and reproduction. The individual steps of developmental toxicity are marked gray. Drugs and chemicals may interfere with each step of the mammalian reproductive cycle, while developmental toxicants may interact with the development of the embryo from fertilization to pre- and postnatal development.

important for male and female fertility. Reproductive toxicity refers to the adverse effects of a substance on any aspect of the reproductive cycle, including the impairment of reproductive function, the induction of adverse effects in the embryo, such as growth retardation, malformations, and death. All these events and interactions are controlled to a large extent by the body's endocrine system. Due to the complexity of the mammalian reproductive cycle, it is not possible to model the whole cycle in one in vitro system in order to detect chemical effects on mammalian reproduction. However, the cycle can be broken down in its biological components that can be studied individually or in combination (Fig. 1). This has the advantage that target tissues/organs of interest can be identified.

During the past 30 years, research in reproductive toxicology has focused on the use of alternatives to living mammals for testing the potential embryotoxicity of chemical and physical agents. Major attention has been devoted to develop and validate in vitro assays for embryotoxicity testing, in particular, test covering the development of the embryo during organogenesis, reflecting important concerns for teratogenic chemicals. Other areas (fertility, implantation, placenta and fetal toxicity) received less attention. In the past, experts have concluded that the use of in vitro methods is well established for conducting mechanistic studies and that such advanced methods already play a valuable role in so-called 'secondary testing', that is, in the screening of a series of structurally related chemicals, when at least one of the chemicals is of known reproductive toxicity in vivo (Brown et al., 1995; Spielmann, 1998).

Developmental toxicity—in vivo tests for regulatory purposes

Currently, multi-generation studies have to be conducted to provide information on the effects of industrial chemicals on all aspects of the highly complex reproductive cycle (OECD, 1983). While for chemicals used as drugs, 'segment studies' have to be conducted covering three important phases of pre- and postnatal development and fertility (ICH, 1994). Due to the complexity of the reproductive cycle, testing in living animals is the only option currently available for assessing the possible effects of chemicals on reproduction including prenatal development. Moreover, due to the complexity of functions that are only found in living animals, in vitro screening may never be able to cover all of the aspects of prenatal development. Thus, the key question is whether sufficient information can be derived from alternative tests to be able to classify and label chemicals as toxic to the developing embryo.

Developmental toxicity—in vitro tests

Over the past 30 years, a wide spectrum of culture systems have been proposed as tests for developmental toxicity. However, the majority of these have been used only in a few laboratories and none of them has been accepted for regulatory purposes. In vitro test for developmental toxicity falls into four categories: established cell lines including embryonic stem cells, primary cell cultures, non-mammalian embryos and mammalian embryos or primordia.

Embryos of lower order species

Although avian embryos are widely used as models in developmental biology, they have rarely been used for embryotoxicity testing. A critical review of the results obtained with the chick embryotoxicity screening test (CHEST) (Jelinek et al., 1985) showed that the test cannot distinguish general toxicity from specific developmental effects (Brown et al., 1995). Of the non-avian vertebrate systems available, only the frog system FETAX (Frog Embryo Teratogenesis Assay) has undergone limited validation using about 40 different substances (Bantle et al., 1990). The overall accuracy in predicting teratogenic potential has been claimed to be 79–83%. FETAX is low-cost and rapid, and uses a species commonly maintained under laboratory conditions. The assay is limited by the aqueous solubility of test substances, by the relative lack of validation and by the small number of laboratories that have used the system. An expert peer review of the performance of the FETAX assay by the US National Toxicology Program NTP and ICCVAM, the US Interagency Coordinating Committee for the Validation of Alternative Methods, concluded that FETAX is not sufficiently vali-