Reproductive Toxicology 49 (2014) 117–135

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Review

Use of ovary culture techniques in reproductive toxicology

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ARTICLE INFO

Article history:
Received 16 May 2014
Received in revised form 14 July 2014
Accepted 6 August 2014
Available online 19 August 2014

Keywords:
Ovary
Follicle
Tissue culture
In vitro
Toxicology
Pharmaceutical
EDC

ABSTRACT

There is increasing evidence to indicate that a substantial number of both man-made and naturally occurring chemicals are disruptive to human and wildlife reproductive health. Currently, reproductive toxicology testing is primarily carried out *in vivo*, however, in the past 50 years, various culture methods have been developed with the aim of growing ovarian follicles *in vitro*. These culture systems have become a widely used tool in reproductive biology and toxicology. In this review we describe how reproductive toxicology of the ovary is greatly enhanced by *in vitro* studies. Experiments using *in vitro* ovarian cultures to understand or detect damage to the ovary itself and to its specialised structures of the follicles and oocytes, allows for faster screening of potential developmental and/or reproductive toxicants.

Contents

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http://dx.doi.org/10.1016/j.reprotox.2014.08.001

1. Introduction

1.1. The ovary

The ovary is central to female reproductive function, the site within which germ cells form follicles, develop and mature (Fig. 1). These cells communicate with each other and with ovarian stromal cells. Mammalian oocytes develop from primordial germ cells during gestation [1]. Following the proliferative stage, primordial germ cells then enter a pre-meiotic state of DNA replication before entering prophase I of meiosis. They then progress through the initial stages of meiosis, before entering meiotic arrest, around the time of follicle formation (Fig. 2) [2,3]. The oocytes remain in this meiotically arrested state throughout the phase of follicular development, thus the growing ovarian follicle contains an oocyte arrested in prophase I of meiosis, surrounded by somatic cells (granulosa and theca cells) as well as a basement membrane (BM). In the postpubertal ovary in particular, as an oocyte grows and matures, its follicle undergoes changes due to proliferation of the granulosa cells and formation of the fluid filled antral cavity, resulting in a dramatic increase in follicle size. Once it has reached full maturation, the pre-ovulatory, Graafian follicle expels its oocyte during ovulation, at which point the oocyte exits meiotic arrest and completes meiosis I.

The ovary is not only responsible for producing oocytes, but is also an important endocrine gland, the source of sex steroids which link reproductive and non-reproductive organs to the timing of the ovarian cycle. It is in the growing follicle that the majority of estrogens in the body are produced and once the oocyte has been ovulated, the remainder of the follicle becomes a corpus luteum (CL), a temporary endocrine structure secreting the progesterone critical for the establishment and initial maintenance of pregnancy. The ovary is responsive to hormones secreted from the anterior pituitary, in turn controlled by the hypothalamus, with which it is locked into a complex cyclical pattern of communication and feedback that underpins successful female reproduction. Ovarian follicles are dependent on both external and internal hormones for their growth, development, maturation and ovulation. Follicle growth through the primordial, primary and secondary stages is gonadotropin-independent, regulated primarily by oocyte-derived factors such as growth differentiation factor 9 (GDF9), bone morphogenetic protein 15 (BMP15) and local somatic-derived factors such as anti-Mullerian hormone (AMH) [4]. Once a follicle transits into the pre-antral stage, its growth to the early-antral stage is gonadotropin responsive, with further growth dependent on gonadotropins [5].

In recent years, both environmental and synthetic pharmaceutical compounds with endocrine-mimicking, -modulating or -inhibiting ability have become an increasing health concern. These compounds have reported harmful effects on gamete development and on the developing foetus and neonate [6]. Their relevance to human pregnancy has been identified in a recent opinion paper from the Royal College of Obstetricians and Gynaecologists (RCOG) which also highlighted the issue of exposure to multiple sources of chemicals. That paper outlined the potential reproductive hazards associated with exposure to the developing foetus of chemicals with the potential to interfere with foetal germ cells in the developing ovary, the effects of which would only manifest in the F2 generations, thus not becoming evident until decades later [7].

Various toxicological studies have been carried out on different animal models both *in vivo* and *in vitro* in order to investigate their effects on the female reproductive system [8]. Both types of toxicology testing have advantages and limitations, which must be carefully considered when planning a toxicological study. Here, we review the role of *in vitro* studies in the examination of toxicological effects on the ovary, comparing results from such methods to the effects of 'real-life', *in vivo*, exposure.

2. Toxicology and reproductive function

The environmental toxicants and pharmaceuticals of concern regarding reproductive function are from a broad spectrum of chemicals. One group in particular, endocrine disrupting compounds (EDCs), constitute a major focus. EDCs have been described by the United States Environmental Protection agency (USEPA) as agents that 'interfere with synthesis, secretion, transport, binding or elimination of natural hormones in the body that are responsible for maintenance of homeostasis, reproduction, development and/or behaviour' [9]. Pharmaceutical and chemical companies produce novel chemicals in the form of new drugs, and widely used industrial and agricultural compounds, which can, in some cases, act as EDCs [10]. Humans are exposed to thousands of these natural or man-made chemicals throughout their lifespan [6,10–12]. Some are ingested as drugs or absorbed through the skin *via* beauty products such as soaps and perfumes [10], whereas others can leach out of plastic or be inhaled from cigarette smoke, pollution or vehicle exhausts (Table 1).

There is increasing evidence suggesting that certain pharmaceuticals and EDCs have the potential to interfere with endocrine function, biosynthesis or homeostatic control, alter reproductive development and fertility and result in reproductive disorders [13,14]. Reproductive toxicants can interfere with endocrine mechanisms due to their weak intrinsic hormonal activity, most often by mimicking or inhibiting estrogens through binding to nuclear, membrane, neurotransmitter and/or orphan receptors.