Preface

In this book, the authors present current research in the study of germ cells. Topics include the molecular signature and sub-cellular machinery of metazoan gametogenic stem cells; recent advances in understanding germ cell-specific organelles and their functions; low-dose radiation induces apoptosis in male germ cells of mice and attenuates it in type 1 diabetes rats; oocyte nuclear structure during mammalian oogenesis; and chromatoid body assembling and nuclear cycles.

Chapter I – In organisms with exclusively sexual reproduction, primary germ cells become segregated during embryogenesis, and only germ cells are responsible for the reproduction. However, embryonic germline segregation is not universal in animal kingdom. In animals with asexual reproduction, the line of pluripotent stem cells is maintained continuously throughout the life of an individual or a colony, giving rise to germ cells and all the types (or a wide spectrum) of somatic cells and so providing a cell source for gametogenesis, asexual reproduction and regeneration. Examples of the gametogenic pluri/multipotent stem cells include sponge archaeocytes, cnidarian interstitial cells, planarian neoblasts, ascidian hemoblasts and stem cells of colonial rhizocephalans. Pluripotent gametogenic stem cells share the evolutionarily conserved features of their morphological and functional organization with germline cells. Pluripotent cells as well as germline cells can be identified by the presence of granular/fibrillar material named nuage or germ granules as a key organelle and a cytoplasmic marker of gametogenic cells across the animal kingdom. Over the years the authors discovered and studied the colonial organization in *Peltogaster reticulatus* and *Thylacoplethus isaevae,* members of the Crustaceans (Rhizocephala). They also indentified vasa-like protein expression as a cytoplasmic marker in the gametogenic stem cells of colonial *Polyascus polygenea* and *Peltogasterella gracilis*. It is believed that germ granules act as a cytoplasmic RNA depot of the germ line cells and are actively involved in RNA processing, silencing and degradation. Germ granules contain products of evolutionary conserved genes, including proteins, mRNAs, and noncoding RNAs. The authors also previously reviewed the macromolecular machinery and network, as well as the molecular signature, of the gametogenic cell across Metazoa, including a set of homologue proteins for Vasa, Piwi/Aubergine, Nanos, Tudor, Pumilio and others. In many species, ranging from sponges and cnidarians to flatworms, annelids, some crustaceans, and chordates, primordial germ cells and pluri/multipotent stem cells are characterized by the expression of a similar overlapping set of genes operating in both gametogenic stem cells types. This conservation

led to the hypothesis that germline and pluripotent stem cells share a common gene regulatory program underlying pluripotency and gametogenic potentiality. These cells appear to actively repress differentiation programs and maintain developmental potency through transcriptional and mitotic quiescence. The evolutionary conserved gametogenic program involves *vasa/pl10*, *piwi/auberdine*, *nanos*, *tudor*, some other genes and signaling ways. Pluripotent gametogenic cells are also similar in their potential and their molecular signature to mammalian embryonic stem cells. Recent data indicate the broad and partially overlapping spectrum of gene expression in embryonic stem, germline, and pluri/multipotent stem cells, in particular, the possible inducibility of germline cells *de novo* without continuous expression of molecular markers of the germ line. In the review, the authors consider some common principles in the sub-cellular and molecular machinery maintaining gametogenic potentiality in germ and gametogenic pluripotent cells in comparative and evolutionary aspects. They postulate evolutionary conserved molecular mechanisms underlying gametogenic potentiality and pluripotency and suppose that pluripotent stem cells, germline cells and embryonic stem cells share a basic molecular machinery as a core regulatory gene networks overlapping to a large extent, which operates in a similar manner across Metazoa, from sponges to chordates, and might be recruited by other tissue-specific networks ensuring self-preservation as a defense mechanism against apoptosis, cell differentiation and aging.

Chapter II – In sexually reproducing multicellular organisms, germ cell fate is specified in early embryogenesis, and the resulting germ cells differ structurally, mechanistically and functionally from the somatic cells. In the majority of animals, germ cells contain a subset of distinct organelles and molecules, which attributes and polar and/or asymmetrical distributions are fundamental for the proper function of the gamete, for fertilization and for the subsequent development and patterning of the embryo. Some of these organelles are shared among many different species, while some are unique and have been specifically acquired or adjusted to fit species-specific developmental requirements. In this review, the authors describe the current state of knowledge on the structures, dynamics and the role of germ cell-specific organelles, molecules, pathways and polarities, and they provide an overview on the most recent insights into their intraspecific uniqueness and interspecific similarities.

Chapter III – Testes are one of the most radiosensitive organs. The loss of male germ cells after exposure to ionizing radiation has been attributed to apoptosis. Low dose radiation (LDR) in the dose range of 25–200 mGy induced significant increases in apoptosis in both spermatogonia and spermatocytes in mice, with the maximal effect at 75 mGy. The increased apoptosis is most likely associated with Trp53 and Fas protein expressions. Furthermore, 75 mGy pre-irradiation led to an adaptive response of seminiferous germ cells in mice to subsequent high dose radiation (HDR)-induced apoptosis. LDR could induce testicular spermatogenic cell apoptosis through mitochondrial- dependent pathway. After male mice were irradiated with LDR, oxidative stress production and several parameters of mitochondrial dysfunction including Na^+-K^+-ATPase activity, $\Delta\psi m$ and mitochondrial substructure in testicular spermatogenic cells, and additionally Cyt c and AIF mRNA and protein expressions, procaspase-9 and -3 protein expressions, caspase-9 and -3 protein cleavages developed. LDR could induce endoplasmic reticulum stress (ERS) in mouse testicular spermatogenic cells, which was regulated by IRE1, PERK and ATF6 pathways, and ERS apoptotic signaling JNK, caspase-12 and CHOP molecules were initiated. LDR could attenuate Type 1 diabetes-induced testicular spermatogenic cell apoptosis in rats, shown by

the decreased mitochondrial potential, increased expressions of Bax mRNA and protein, not changed serum sex hormones and decreased serum and testicular oxidative damages. These results suggest that LDR induces increased apoptosis in male germ cells through three major pathways: the pathways of receptor-mediated spermatogenic cell apoptosis via death receptor of Fas/FasL and p53 signaling, mitochondrial damage and caspase cascade, and ERS and caspase cascade, but also induces a significant adaptive response that decreases spermatogenic cell death after a subsequent HDR; LDR protection from diabetes-induced testicular spermatogenic cell apoptosis is most likely mediated by its preserving antioxidants.

Chapter IV – Structure and function of the mammalian oocyte nucleus (germinal vesicle, GV) are reviewed in the light of the concept of nuclear architecture and compartmentalization. Some peculiarities of oocyte nuclear architectonics in different mammalian species are compared. Structural changes of chromatin configuration during mammalian oogenesis are discussed with special emphasis on the karyosphere formation. Besides, special attention is paid to the structure, dynamics and molecular composition of the nucleolus, nucleolus-derived structures, and non-nucleolar extrachromosomal domains in the mammalian GV.

Chapter V – This study aimed to find physiological, morphological, and/or structural relationship between two important physiological events that take place during vertebrate spermatogenesis: the nucleolar cycle and chromatoid body (CB) assembly. Both process were analyzed by cytogenetics and ultrastructural analysis, which were carried out in adult male of four vertebrate species: *Tilapia rendalli* (Teleostei, Ciclidae), *Dendropsophus minutus* (Amphibia, Anura), *Phrynops geoffroanus* (Reptilia, Testudines), and *Oryctolagus cuniculus* (Mammalia, Lagomorpha). The authors also determined the number of nucleoli contained in spermatogonia and earlier/round spermatids, besides of performing measurements of the ratio between nuclear and nucleolar areas of spermatogonia and earlier/round spermatids. They found minor differences in some aspects of both nucleolar cycle and chromatoid body assembly, such as nucleolar architecture, timing of nucleolar disruption at first meiosis division, timing of CB assembly in the cytoplasm of germ cells, CB association to other structures, and its positioning in the cytoplasm of earlier/round spermatids. In general, there was no alteration regarding the number of organized nucleoli in the nucleus of cells undergoing through meiotic division. All species had generally one single organized nucleolus in both germ cell types, with an exception to *O. cuniculus*, which showed a higher number of germ cells with 2, 3, or 4 organized nucleoli. There was an increase in the nuclear and nucleolar area ratio mean between diploid spermatogonia and haploid earlier/round spermatids in all species, mainly in *T. rendalli* and *P. geoffroanus*. This increase is probably related to the decrease in the nucleolar size after germ cells undergo the meiotic division. Many factors could contribute to the reduction of nucleolar area after meiosis, one of them being the probable migration of the nucleolar fragments to the cytoplasm of germ cells in prophase I, where they may play a role in CB assembling, as previously proposed. Former detection of nucleolar proteins in the chemical composition of the CB is a strong evidence supporting this idea. If nucleolar proteins would play a role in the CB physiology acting in the RNA processing or if they would be translocated to the CB, because they are targeted for degradation in late steps of the spermatogenesis process, are further questions that still need to be investigated. However, the evidences showed in this study point for the existence of a physiological, morphological, and/or structural link between the CB assembly and the nucleolar cycle in the spermatogenesis process of vertebrates.