Animal Reproduction Science xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Animal Reproduction Science

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

# Structure of nucleoli in first-order spermatocytes of selected free-living animal species

Katarzyna Andraszek[a,*], Magdalena Gryzińska[b], Mariola Ceranka[a], Agnieszka Larisch[a]

[a] Department of Animal Genetics and Horse Breeding, Institute of Bioengineering and Animal Breeding, University of Natural Sciences and Humanities, 14 Prusa Str, 08-110 Siedlce, Poland
[b] Department of Biological Basis of Animal Production, University of Life Sciences, Lublin, Akademicka 13, 20-950 Lublin, Poland

ARTICLE INFO

*Article history:*
Received 23 February 2015
Received in revised form 17 June 2015
Accepted 19 June 2015
Available online xxx

*Keywords:*
Roe deer
Wild boar
Spermatogenesis
Nucleolus
Chromatoid bodies

ABSTRACT

Nucleoli are the product of the activity of nucleolar organizer regions (NOR) in certain chromosomes. Their main functions are the formation of ribosomal subunits from ribosomal protein molecules and the transcription of genes encoding rRNA. Nucleoli are present in the nuclei of nearly all eukaryotic cells because they contain housekeeping genes. The size and number of nucleoli gradually decrease during spermatogenesis. Some of the material originating in the nucleolus probably migrates to the cytoplasm and takes part in the formation of chromatoid bodies (CB). Nucleolus fragmentation and CB assembly take place at the same stage of spermatogenesis. CB are involved in the formation of the acrosome, the migration of mitochondria to the midpiece, and the formation of the sperm tail fibrous sheath. The aim of the study was to characterize the nucleoli in the early prophase of spermatogenesis in the wild boar and the roe deer. The roe deer cells have larger nucleoli and a larger cell nucleus than the wild boar cells. The area of the nucleolus as a percentage of the total area of the nucleus was larger as well. The coefficients of variation for all parameters were higher in the roe deer. In the wild boar cells the nucleoli were mainly regularly shaped. The size of the nucleolus and the nucleus of the spermatocyte is a species-specific trait associated with karyotype and the number of nucleolar organizer regions in a given species.



## 1. Introduction

The nucleolus is a structure of the cell nucleus whose main task is ribosome synthesis (Hernandez-Verdun, 2011). Many studies, however, have shown that the nucleolus has functions unrelated to the biosynthesis of ribosomes. It is involved in regulation of the cell cycle, biogenesis of ribonucleoproteins, and cell proliferation. It has been shown to be associated with cancerogenesis, viral infections, degenerative diseases and cellular stress (Gerbi et al., 2003; Khurts et al., 2004; Nafe and Schlote, 2004; Raška et al., 2006; Boisvert et al., 2007).

In the cells of eukariotic organisms nucleoli can have an irregular, oval or spherical shape (Olson and Dundr, 2005). Irregular forms may indicate ageing processes in the cell and the beginning of the apoptosis process (Olson et al., 2002). Smaller nucleoli and micronucleoli indicate irreversible cell damage caused by total and irreversible inhibition of RNA synthesis (Horky et al., 2002). Nucleoli are the least stable organelles in the cells of adult mammals. Their morphological traits depend on many factors. Factors influencing changes in the structure of nucleoli

* Corresponding author.
*E-mail address:* andrasz@uph.edu.pl (K. Andraszek).

http://dx.doi.org/10.1016/j.anireprosci.2015.06.013
0378-4320/

include the species of animal, the type and degree of cell differentiation, the role of a given cell, the physiological or pathological state of the organism, and the phase of the cell cycle (Olson et al., 2002; Hernandez-Verdun, 2011). Epigenetic regulations at the level of the nucleolus and rRNA genes continue to be a key subject of genetic research.

The nucleolus is capable of changing its size, becoming denser, and dissipating during each cell cycle. Its functioning is best known and described in somatic cells (Pikaard, 2002; Raška et al., 2006; Hernandez-Verdun, 2011). In mitosis nucleoli are highly dynamic structures. An expression of this is their cyclic disappearance during mitosis and appearance when mitosis is complete (Scheer and Benavente, 1990). The nucleolus disappears during cell division and is reformed in reconstructed nuclei as a result of NOR activity (Olson et al., 2002). Nucleolar activity appears near NOR-bearing chromosomes when the telophase nucleus begins to reform. Then rRNA synthesis is renewed, making the nucleoli more clearly visible. During interphase the nucleolus has a spherical shape. In prophase, when chromosomes become visible, the nucleolus is associated with particular nucleolus organizing chromosomes (Raška et al., 2006).

During meiosis the nucleolus behaves differently than in somatic cells. They undergo gradual degradation throughout the prophase of the first meiotic division. The manner in which they disappear is probably characteristic for particular groups of vertebrates and may be a species trait. In the spermatocytes of domestic cattle the nucleoli gradually become fragmented and break up into very small structures whose number corresponds to the number of nucleolar organizer regions (Andraszek and Smalec, 2012a). According to a study analysing the number and size of nucleoli in the spermatocytes of birds (Andraszek and Smalec, 2012b) the disappearance of the nucleoli proceeds differently. In birds the reorganization of chromatin during the prophase of the first meiotic division and the associated change in the size of the cell nucleus is correlated with the decreasing size of the nucleoli.

The process of spermatogenesis has been and continues to be the subject of many studies and has been thoroughly described in the literature. There are, however, numerous recently discovered aspects of spermatogenesis that have yet to be researched, such as the mechanisms of the decrease in size of nucleoli during meiosis and their fragmentation and reorganization, or the formation and role of chromotoid bodies (CB), which are functionally associated with the nucleolus. These are typical cytoplasmic organelles present in haploid reproductive cells during spermatogenesis up to the differentiation stage. These studies are supplemented by analysis of the precise position of the nucleolus in the spermatogonium and spermatocyte (Peruquetti et al., 2012).

Many studies have been conducted with the purpose of explaining CB genesis and function. Attempts have been made to identify CB with the protein MVH (Mouse Vasa Homologue), which is dependent on ATP and RNA helicase. This protein is involved in the formation and differentiation of male reproductive cells and has been found to be associated with CB in several species (Kotaja et al., 2006a,b). Another interesting element of CB is the protein MEAL, which is associated only with silencing of sex-determination genes. MEAL takes part in the miRNA pathway and may silence genes located on sex chromosomes forming an incomplete synaptonemal complex (Costa et al., 2006). Some authors suggest that CB is a ribonucleoprotein complex originating in the nucleolus and migrating to the cell cytoplasm during the early stages of spermatogenesis (Peruquetti et al., 2008, 2010). However, these still remain hypotheses.

The nucleolus of reproductive cells plays a major role in the control of meiosis. The nucleolus contains most of the cellular protein Pch2, which detects faulty organization of synaptonemal complexes and prevents segregation of chromosomes during meiotic recombination (Peruquetti et al., 2012).

Basic genetic and cytogenetic research is increasingly being expanded to include evolutionary research, mainly concerning the evolution of genomes. In this type of research access to reference material – the genome of the ancestor – is invaluable. This is preferably a free-living species whose genome has not yet been interfered with by human beings, or in which the interference has been minimal. Such species include the roe deer and the wild boar, the wild ancestors of domesticated small ruminants and pigs. They supply invaluable material for comparative research in cytogenetics, epigenetics and comparative genomics.

The aim of the study was to characterize the number, structure and morphometry (area, perimeter, length and width) of nucleoli and to determine the percentage share of the nucleolus in the total area of the cell nucleus in the early prophase of spermatogenesis in selected species of free-living animals.

## 2. Materials and methods

The study was carried out according to the guidelines of the III Ethical Committee in Warszawa (No 37/2011 from the 22 June 2011).

The study was conducted on wild boar (*Sus scrofa*) and roe deer (*Capreolus capreolus*). Testicular biopsies were taken *post mortem* from 10 males of each species. Nucleoli of primary spermatocytes isolated according to Evans et al. (1964) were analyzed. Fixed cells were suspended in a small volume of fresh Carnoy's solution, spread over degreased, cold microscope slides, and air-dried at room temperature. The specimens were stained according to a method by Howell and Black (1980), and then immersed for a few seconds in Giemsa solution. The prepared specimens were evaluated under an OLYMPUS BX 50 microscope. The microscope images were stored in computer memory using MultiScan software and a digital Olympus DP25 camera. The images were analyzed using graphics and statistics software integrated with the MultiScan system. From each individual 20 primary spermatocytes in the early meiotic prophase were analyzed. The number and shape of the nucleoli were evaluated and the nucleoli and the cell nucleus of the spermatocyte were characterized morphometrically. Measurements were made of the area, perimeter, length and width of the nucleolus and the cell nucleus, as well as the percentage share of the nucleolus in the total surface area of the cell nucleus. The nucleoli were

Please cite this article in press as: Andraszek, K., et al., Structure of nucleoli in first-order spermatocytes of selected free-living animal species. Anim. Reprod. Sci. (2015), http://dx.doi.org/10.1016/j.anireprosci.2015.06.013