Observational study

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Unraveling the Mystery of Multi-Oocyte Follicles: An Observational Study

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Abstract

Introduction. Significant progress has been made in understanding oocyte fertilization and early developmental stages through in vitro fertilization (IVF) techniques. However, irregularities such as conjoined oocytes and binucleate giant oocytes, which are exceptions to the normal rule of one diploid female gamete per follicle, can potentially lead to chromosomal disorders in embryos and are recommended to be excluded from IVF attempts. The formation of primordial follicles during ovarian development, known as follicle assembly, is a critical process that establishes the ovarian follicle reserve. Multi-oocyte follicles (MOFs) containing two or more oocytes have been observed in various species, including humans, and their clinical significance on fertility and reproductive health remains unclear. Genetic and environmental factors, such as gene knockout and exposure to endocrine disruptors, have been implicated in MOF formation, but the mechanisms are not fully understood and require further investigation.

Material & Method. In this Observational study, 350 slides of ovarian tissues were scanned using an AI-based automated microscope, Grundium Ocus 20, and the TIFF images were stored in cloud storage. The slides were examined using third-party software, Pathcore Seeden Viewer, for morphometry of binovular follicles.

Results. In our observational study, we examined 350 ovarian tissue slides in detail by using an AI-based microscope, uncovering 22 slides from seven different tissues with binovular and multinovular oocytes. These rare multi-oocyte follicles (MOFs) challenge the conventional one-oocyte-per-follicle paradigm. MOFs are likely formed when oocytes fail to separate during cortical sex cord proliferation, regulated by factors several molecular factorsas well as environmental factors.

Discussion. Multiple Ovarian Follicles (MOFs) are rare phenomena where two or more oocytes exist in one follicle. They arise when oocytes fail to separate during ovarian development, which is governed by hormones such as follicle-stimulating hormone (FSH), inhibin, BMP-15, GDF-9, and GCNF. MOFs can be caused by dysregulation and exposure to chemicals such as diethylstilbestrol (DES) and isoflavones. Binovular oocytes, which occur when two oocytes are released during ovulation and are fertilised by different sperm, can result in non-identical twins, which are influenced by genetic and environmental factors such as maternal age, heredity, hormonal imbalances, and assisted reproductive techniques such as in vitro fertilisation (IVF). Polynuclear oocytes with many nuclei can develop as a result of meiotic spindle defects and environmental influences. Identifying these oocyte types may aid in improving ART results by improving knowledge of the reasons of infertility and devising appropriate interventions. ***Clin Ter 2024; 175 (1):42-46 doi: 10.7417/CT.2024.5032***

Keywords: *Binovular, Multi-oocyte follicles, follicle fusion, Granulosa Cell, Ovary*

Introduction

Since the development of human-assisted reproduction techniques, significant progress has been made in understanding oocyte fertilization and early developmental stages. In vitro fertilization has provided insights into irregularities during gamete development. Normally, a follicle should contain only one diploid female gamete, resulting in a haploid secondary oocyte after the first meiotic division. However, conjoined oocytes and binucleate giant oocytes are exceptions to this rule, and they are sporadically observed in in vitro fertilization programs. Binucleate giant oocytes are now recognized as a potential source of chromosomal disorders in the embryo, and it is recommended to exclude them from in vitro fertilization attempts. On the other hand, our knowledge of conjoined oocytes is limited to a few case reports, indicating that some fertilized gametes have been transferred without or after mechanical separation. However, pregnancies resulting from these transfers have not been reported.

The formation of primordial follicles, also known as follicle assembly, is a critical process in ovarian development that establishes the ovarian follicle reserve. This process occurs during midgestation in humans and within the first

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postnatal days in rodents. During early ovarian development, oocytes are initially grouped together in nests surrounded by stromal cells, forming ovigerous cords. However, more than half of the oocyte population undergoes programmed cell death, and surviving oocytes are surrounded by a layer of flattened granulosa cells, resulting in the formation of a finite population of nongrowing follicles (1). Multi-oocyte follicles (MOFs) containing two or more oocytes have been observed in various species, including humans (2-4). While the clinical significance of MOFs on fertility remains unclear, they are thought to be generated by alterations in nest breakdown and/or follicle assembly, making them a potential marker of disruptions in these essential processes. Several genetic and environmental factors, such as knockout mice for different genes and exposure to endocrine disruptors and phytoestrogens, have been implicated in the formation of MOFs.

Recently, unexpected high frequencies of MOFs have been observed in intact peripubertal rats during postnatal development, from the infantile period to adulthood (5). This observation has led to the identification of a new mechanism for MOF generation in rats, which involves the plasticity of ovarian follicles and the atypical behavior of granulosa cells. Further analysis suggests that granulosa cells in the ovaries of peripubertal rats exhibit unique characteristics, deviating from the conventional pattern of follicle assembly. This finding sheds new light on the complex process of follicle assembly and highlights the need for further research to better understand the factors and mechanisms regulating MOF formation in different species, including their potential implications for fertility and reproductive health. The formation of primordial follicles during follicle assembly is a tightly regulated process that involves complex interactions between oocytes and surrounding somatic cells, including granulosa cells (1). The survival and growth of primordial follicles are critical for the establishment of the ovarian follicle reserve, which determines the reproductive potential of females. However, the mechanisms underlying the generation of MOFs, particularly in rats, are not fully understood and require further investigation. The plasticity of ovarian follicles, as observed in rats, is an intriguing phenomenon that challenges the conventional understanding of follicle assembly. Granulosa cells, which are typically flattened and form a single layer around the oocyte in primordial follicles, exhibit atypical behavior in rats, leading to the formation of MOFs (5). This atypical behavior may involve changes in the communication between oocytes and granulosa cells, alterations in cell proliferation or apoptosis, or modifications in the extracellular matrix that surrounds the follicles. Further research is needed to elucidate the underlying mechanisms responsible for this plasticity and the formation of MOFs in rats.

In addition to the plasticity of ovarian follicles, other factors may also contribute to the formation of MOFs. Genetic and environmental factors, such as gene knockout and exposure to endocrine disruptors or phytoestrogens, have been implicated in MOF generation in various species. For example, knockout mice for specific genes involved in follicle development have been shown to exhibit altered follicle assembly and increased MOF formation. Similarly, exposure to endocrine disruptors or phytoestrogens during critical periods of ovarian development has been linked to MOF formation in rodents.

In humans, the ovaries of young women who undergo an ART (assisted reproductive technique) may show MOFs (6). Dysregulation in the proliferation of the cortical cords of developing ovary may lead to these MOFs. It had been reported that downregulation of many factors like FSH/inhibin (7), BMP-15/GDF-9 (8), GCNF (9) which regulates the normal ovarian development may leads to MOFs even the upregulation or overexpression of inhibin-α subunit also shows higher incidence of these MOFs (10). Besides these intrinsic genetic factors, many environmental chemical factors like diethylstilbestrol (11), isoflavone (12), genistein (13), and bisphenol A (BPA) (14,15) influence the formation of MOFs. However, the precise mechanisms by which these factors influence MOF formation are not yet fully understood and require further investigation. The clinical significance of MOFs on fertility and reproductive health remains uncertain. MOFs have been observed in human ovaries, but their impact on fertility and reproductive outcomes is not well established.

Materials & Method

During the routine microanatomy slides preparation process for the teaching of undergraduates, we found the section of many ovaries stained with H&E which had binovular follicles. So, we decided to look at all the slides of ovarian tissue available in the department to see these binovular follicles. Primary aim of the study was to evaluate the occurrence of rare enigmatic phenomena of Binovular, Multi-oocyte follicles. For which we had scanned 350 slide belongs to different ovarian tissues by using an A.I. based automated scanner microscope grundium ocus 20. This microscope scanned the whole tissue section on a slide and store the TIFF image at cloud storage which was analysed by using the 3rd party software pathcore seeden viewer with morphometery of all binovular follicles.

Result

In our observational study, we examined 350 ovarian tissue slides in detail by using an AI-based microscope, On careful examination of the each and every slide we found that 22 slides of different 7 ovarian tissues had binovular as well as multinovular oocytes (Fig. 1).

Maximum width of these binovular follicles were 934 μm (Fig. 2). These rare multi-oocyte follicles (MOFs) challenge the conventional one-oocyte-per-follicle paradigm. MOFs are likely formed when oocytes fail to separate during cortical sex cord proliferation, regulated by factors several molecular factorsas well as environmental factors.

Discussion

Multiple Ovarian Follicles (MOFs) are a rare occurrence in the ovaries and are typically characterized by the presence of two or more oocytes within a single follicle (16).

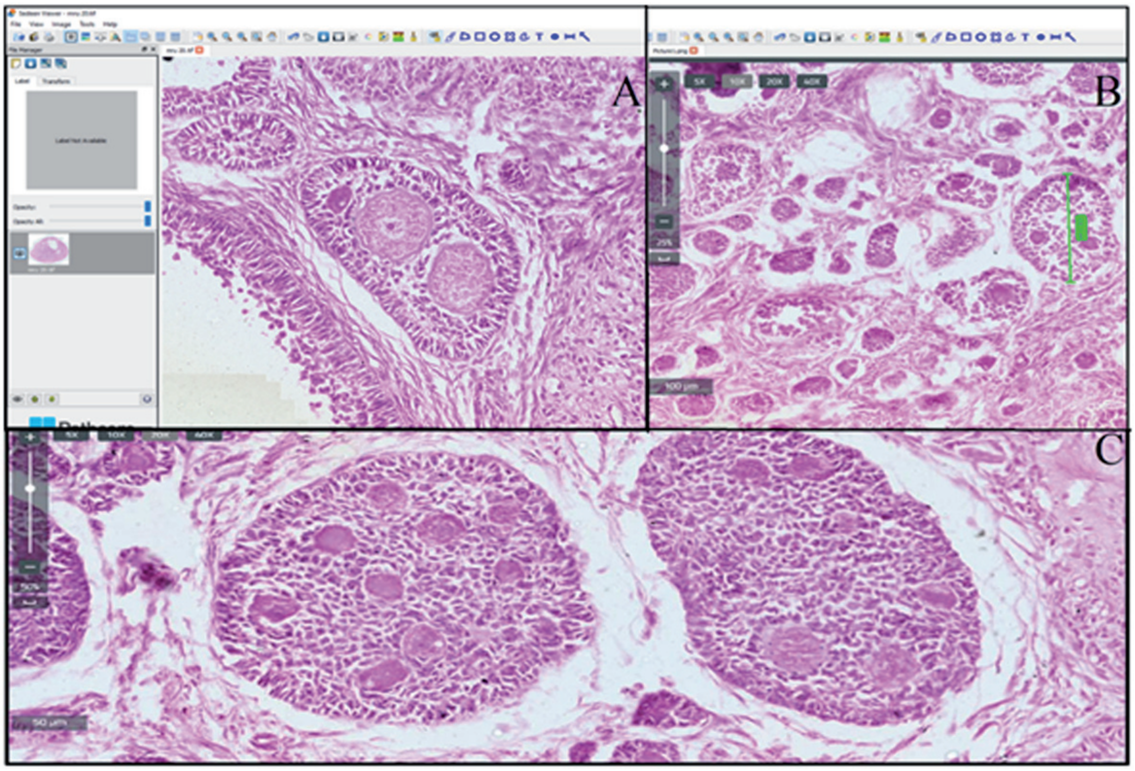

Fig. 1. In section A & B, binovular ovarian follicle is clearly visible and in section C Multi-oocyte ovarian follicles are present.

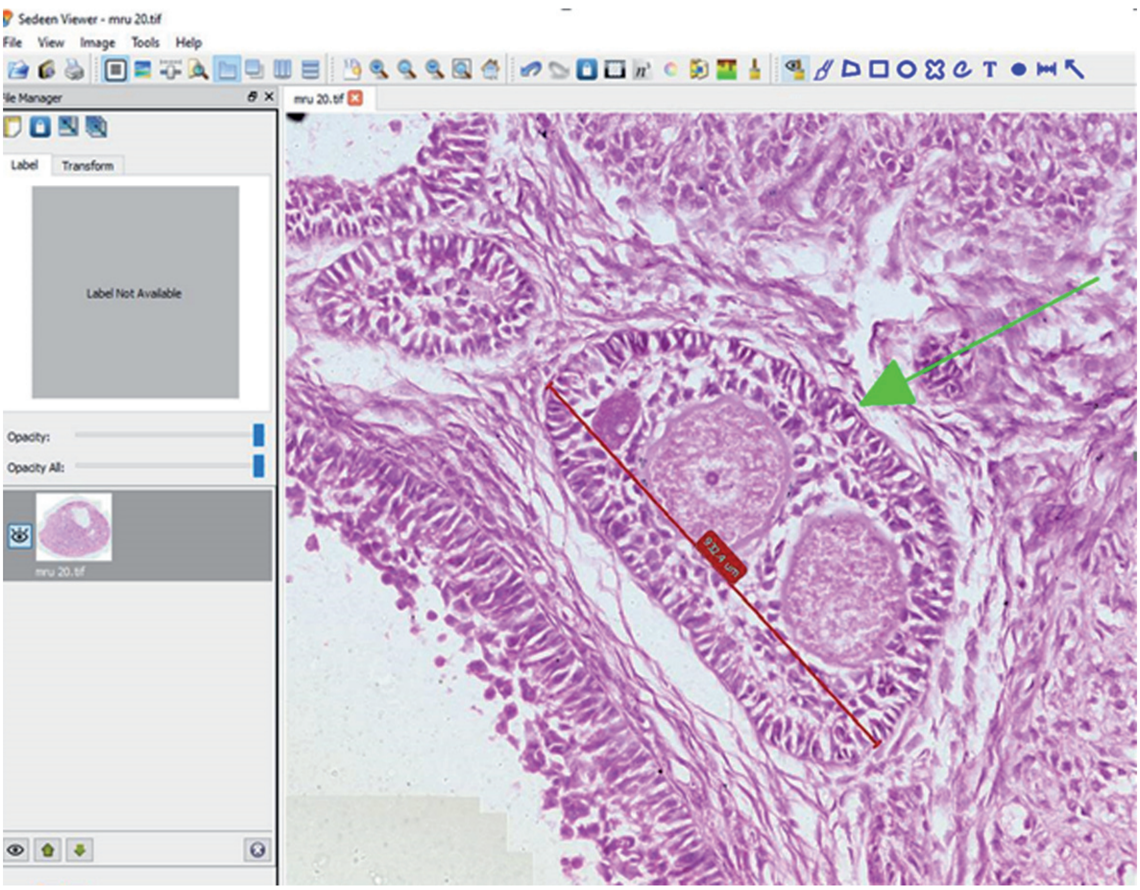

Fig. 2. Size of the all bi & multi-ovular follicles measured by using Seeden Pathcore Viewer software after archiving the images from the GRUNDIUM OCUS®20 (Automated Microscope)

The development of MOFs has been attributed to various factors, including the division of a polynuclear oocyte or the fusion of two or more individual follicles. However, the most accepted cause in current research is that MOFs are formed due to the non-separation of multiple oocytes during the proliferation of cortical sex cords in developing ovaries. This observation is consistent with the findings of Reynaud et al. (2010) and suggests that dysregulation of the proliferation of cortical cords in developing ovaries is the primary cause of MOFs (3). The normal development of ovaries is regulated by several factors, including follicle-stimulating hormone (FSH)/inhibin, bone morphogenetic protein-15 (BMP-15)/growth differentiation factor-9 (GDF-9), and germ cell nuclear factor (GCNF). Downregulation of these factors has been implicated in the formation of MOFs. For example, overexpression or upregulation of inhibin-α subunit has been shown to result in a higher incidence of MOFs.

In addition to genetic factors, environmental factors have also been reported to influence the formation of MOFs. One such environmental factor is diethylstilbestrol (DES), a synthetic estrogen that was commonly prescribed to pregnant women to prevent miscarriages. However, it was later discovered that DES exposure in utero increased the risk of developing various reproductive tract abnormalities, including MOFs. DES exposure during fetal life has been shown to cause persistent alterations in ovarian structure and function, leading to an increase in the number of oocytes per follicle. DES exposure has also been shown to affect the expression of several genes that regulate folliculogenesis, including BMP-15, GDF-9, and follicle-stimulating hormone receptor (FSHR), which may contribute to the development of MOFs. Isoflavones are plant-derived compounds that are structurally similar to estrogen and are found in various foods, with soy products being a common source. Several studies have reported that high intake of soy products during early life increases the risk of MOFs in adulthood. Isoflavones are known to bind to estrogen receptors and affect estrogenic signaling pathways. Genistein, a major isoflavone in soy, has been shown to interfere with the normal ovarian development of rats by altering the expression of several genes that regulate folliculogenesis (12). MOFs are believed to be primarily caused by the non-separation of multiple oocytes during the proliferation of cortical sex cords in developing ovaries. Dysregulation of factors such as FSH/inhibin, BMP-15/GDF-9, and GCNF, as well as exposure to environmental factors such as DES and isoflavones, may contribute to the development of MOFs.

The concept of binovular and polynuclear oocytes, though not widely known, is not uncommon in the field of reproductive medicine. Several molecular and environmental factors are believed to contribute to the occurrence of these unique oocyte characteristics, and their proper identification and examination are crucial in assisted reproductive technology (ART) and infertility centers. Binovular oocytes, also known as dizygotic or fraternal twins, are formed when two separate oocytes are released from the ovary during ovulation and are fertilized by two different sperm, resulting in the development of two embryos. This can lead to the birth of non-identical twins with different genetic makeup and physical characteristics. Binovular twinning has been linked to both genetic and environmental factors, including maternal age, heredity, and hormonal imbalances. Recent research has also suggested a possible role of epigenetic factors and assisted reproductive techniques, such as in vitro fertilization (IVF), in the occurrence of binovular oocytes. Polynuclear oocytes, on the other hand, are oocytes that contain more than one nucleus. This phenomenon, also known as polykaryocytosis or multi-nucleation, can occur during the maturation process of the oocyte in the ovary. Polynuclear oocytes have been associated with various molecular factors, including abnormalities in the meiotic spindle, cytoskeleton defects, and mitochondrial dysfunction. Environmental factors such as exposure to toxins, stress, and hormonal imbalances may also play a role in the occurrence of polynuclear oocytes.

In ART and infertility centers, the identification and examination of retrieved oocytes are crucial for successful outcomes. The detection of binovular or polynuclear oocytes requires careful examination under a microscope during the oocyte retrieval process. Proper identification of these unique oocyte characteristics is essential to avoid potential risks and complications in ART procedures, such as incorrect embryo selection, misdiagnosis of genetic abnormalities, and reduced success rates. Furthermore, understanding the underlying molecular and environmental factors associated with binovular and polynuclear oocytes can provide valuable insights into the complex processes of oocyte development and maturation. This knowledge can help researchers and clinicians better understand the causes of infertility and develop strategies to improve ART outcomes. There were certain limitations in the study like small sample size & patient history. As it is very difficult to get the normal ovarian tissue for section and analysis, lot of ethical consideration has to be follow.

Conclusion

The concept of binovular and polynuclear oocytes may not be widely known, they are not uncommon in the field of reproductive medicine. Molecular and environmental factors are believed to contribute to their occurrence, and proper identification and examination of retrieved oocytes are crucial in ART and infertility centers. Further research and understanding of these unique oocyte characteristics can contribute to advancements in reproductive medicine and improve the success rates of ART procedures.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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