We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Chapter 6

The Primordial to Primary Follicle Transition — A Reliable Marker of Ovarian Function

Duda Malgorzata, Grzesiak Malgorzata, Knet-Seweryn Malgorzata and Zbigniew Tabarowski

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62138

Abstract

In many mammalian species, including humans, folliculogenesis begins in fetal life and progresses throughout adulthood. The growing follicles progress from a reserve of primordial follicles that constitute the pool of female gametes for the entire life. Primordial follicles may begin to grow either immediately after forming or at clearly defined species-specific gap. Alternatively, some follicles may become quiescent before they either degenerate or resume growth several months or years afterwards. The rate of follicular assembly and the primordial to primary follicle transition is a critical step in female fertility. Therefore, disturbed coordination of the formation of primordial follicles and activation of their growth may entail some reproductive disorders. A poor initial reserve or the precocious primordial follicle depletion will result in infertility that, in women, is escorted by a shortened reproductive lifespan and early menopause. Therefore, it seems necessary to reach a profounder understanding of the molecular and cellular mechanisms controlling follicular development during preantral transition. *In vitro* growth of isolated immature ovarian follicles (IVGF) appears as an emerging technology, allowing to expand the fertility options in particular ovarian disorders or after cancer treatment

Keywords: Preantral follicles, folliculogenesis, organ cultures, *in vitro*, growth of isolated immature ovarian follicles (IVGF)

1. Introduction

In the ovary of a mammalian female, the process of folliculogenesis begins during fetal life and proceeds until the end of reproductive capacity, which is manifested in cell proliferation and differentiation [1,2]. Folliculogenesis, involving growth and development of ovarian follicles from primordial to preovulatory stages, is a complex phenomenon requiring multidirectional

INTECH
open science | open minds

© 2016 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

regulation. The ovarian follicle plays an essential role in securing optimal conditions for oocyte maturation and its release during ovulation, for which it will provide an appropriate microenvironment based on locally produced molecules, such as sex steroids and peptide hormones, growth factors, and cytokines, while also providing the appropriate communication among particular compartments of an ovarian follicle [3–5]. Sex steroids produced by follicular cells are known to play one of the main roles in the regulation of ovarian function [6]. These steroids present in the systemic circulation actively participate in the regulation of pituitary gonadotropin secretion. On the other side, sex steroids present in the ovarian microenvironment act as paracrine factors important for the maintenance of follicular development [3]. The majority of information about the role of sex steroids in ovarian functioning has been obtained in studies directed at the action of estrogens [7] and progestagens [8]. Nowadays, increasing attention is being devoted to the action of androgens because the activation of androgen receptors (ARs) located in follicular cells [9,10] modulates the expression and activity of many genes vital for the maintenance of follicular development [11–13].

From the initial pool of ovarian follicles recruited to grow, only a few reach a preovulatory stage. Less than 1% of follicles elude the process of atresia at various stages of development, and the preantral to early antral transition is the most susceptible to this process. The pool of primordial follicles established in fetal life constitutes a reserve that will not increase during the postnatal period. The initial stages of folliculogenesis, including the accumulation of primordial follicles, the recruitment of primordial follicles from the resting pool, and their transition into primary follicles, are crucial for the female reproduction regardless of the species [14]. Improper coordination of the formation of primordial follicles and activation of their growth may entail disturbed folliculogenesis in mature individuals manifested by a reduction of fertility. Recent research has revealed that primordial and primary follicles might not die by classical apoptosis. It is therefore possible that, in the immature ovary, other mechanisms are involved in follicular atresia [15].

The main factor determining the selection of follicles into the antral stage is their ability to respond to gonadotropins, especially follicle-stimulating hormone (FSH). Preantral follicles display an increase in the number of FSH receptors (FSHR) that, when activated, stimulate granulosa cell proliferation, antrum formation, and biosynthesis of estradiol after the activation of aromatase enzyme. There is quite ample evidence that follicle development is dependent on their granulosa layer, the functioning of which is influenced by endocrine, paracrine, and autocrine mechanisms. Granulosa cells are involved in the control of oocyte maturation and proper execution of ovulation and participate in early embryogenesis, maintenance of corpus luteum function, and production of chemotactic factors and those involved in angiogenesis [16]. Sustained oocyte growth depends on the effective communication and crosstalk between granulosa cells and the oocyte, because granulosa cells remain the major source of nutrients for the gamete through homologous and heterologous gap junctional contacts [17].

The tool that allows studying the function of ovarian follicles irrespective of its complicated structure is the model of whole organ culture, which reflects the conditions and complicated interactions occurring *in vivo*. These kinds of cultures constitute very sensitive objects to test the biological activity of various factors; they allow to observe the responses to increased or

http://dx.doi.org/10.5772/62138

decreased steroid hormone secretion, the induction or inhibition of cell proliferation, or the induction or inhibition of apoptosis. The technological revolution in reproductive biology that started with artificial insemination and embryo transfer technologies during the last 30 years has continued with oocyte *in vitro* maturation (IVM), *in vitro* fertilization (IVF), or *in vitro* embryo culture (IVC), to name only a few. IVM has particular significance, providing the technology platform for the abundant supply of mature, good quality oocytes for diverse applications, such as reducing the generation interval in important species or studying *in vitro* human reproduction. Despite the convenience of IVM, we still do not understand the precise factors and conditions occurring *in vivo*, which yield the highest-quality mature oocytes for successful fertilization and embryo development outcomes; hence, we cannot completely imitate these conditions. Thus, *in vitro* growth of isolated immature ovarian follicles (IVGF) appears as an emerging technology allowing to expand the fertility options, particularly in young cancer patients [18–20], and may serve as a potential source of fertilizable gametes. Thus, assisted reproductive technologies allied to a profound understanding of granulosa/oocyte interactions can benefit from the capability to sustain primordial and primary follicle growth *in vitro* while supporting the acquisition of oocyte competence.

On this basis, the objective of this chapter is to review relevant data concerning the molecular factors crucial to the regulation of early stages of folliculogenesis and to provide basic information to the design of future culture strategies promoting the *in vitro* development of ovarian follicles.

2. Development of the primordial follicle

In the mammalian embryo, ovarian development begins between 3 and 6 weeks after conception. During this period, ovarian rudiment is massively colonized by mesonephric cells, which are regarded as the follicular cell precursors, and the primordial germ cells (PGC) migrate into the genital ridge; hence, other events take place, such as the differentiation of the gonads according to gender, proliferation, and apoptosis [14,21,22]. Oocyte development begins in the mammalian female fetus together with the differentiation of PGC. Proliferating PGC migrate towards the nascent genital ridges, where they differentiate into oogonia, before entering the first meiotic division to become primary oocytes [26].

Mammalian oocytes develop and reach ovulatory maturity inside the follicles where they are covered at first by pre-granulosa and then by granulosa cells [23] (Figure 1). Over the lengthy process of follicle development, granulosa cells proliferate and the theca layer is formed [24], allowing the follicle to take advantage of blood supply. Then, follicles pass through the succeeding stages of development before reaching full maturation and the ability to ovulate [25]. Primary oocytes, which are arrested at diplotene of the first meiotic prophase since late prenatal life in most mammal species, are the organizing centers of primordial follicles. The oocyte is considered to play the most important role in follicular organization during folliculogenesis. It is assumed that the oocyte controls both the proliferation and the differentiation of granulosa cells into cells capable of secreting steroids and various proteins. On the contrary, several oocyte features, such as growth, differentiation, meiosis, cytoplasmic maturation, or

control of transcriptional activity, are dependent on the presence and contact with granulosa cells [27]. Interestingly, when the oocyte reaches a certain size threshold, it secretes factors that inhibit the ability of granulosa cells to promote its own growth [4], which suggests that the oocyte may determine not only its own growth but also the growth of the whole follicle.

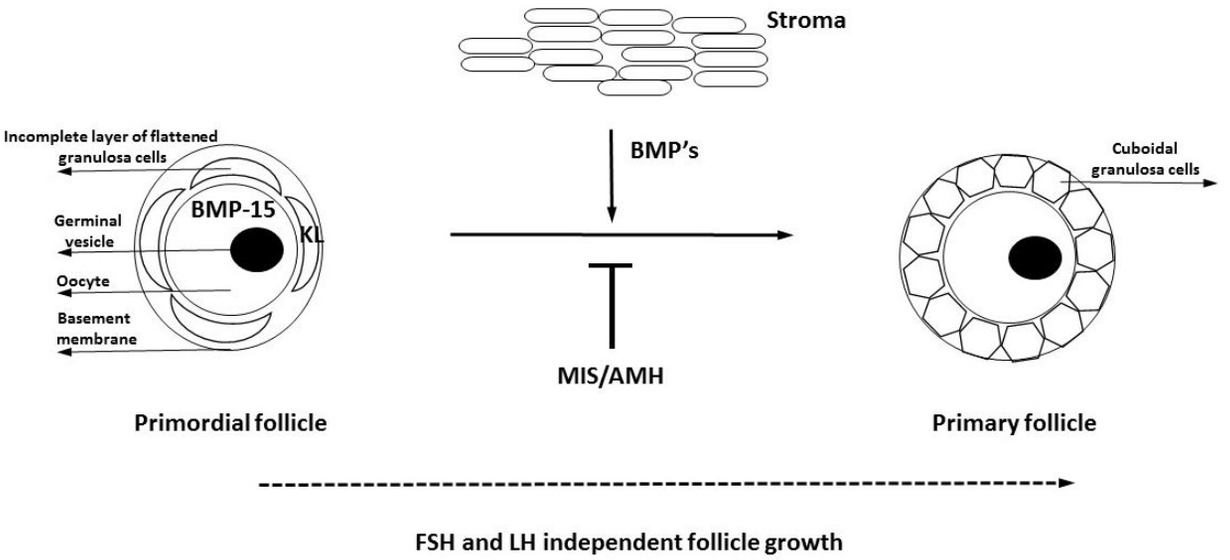


Figure 1. Simplified representation of early stages of mammalian folliculogenesis. KL, kit ligand; BMPs, bone morphogenic proteins; MIS/AMH, Müllerian inhibitory substance.

The assembly of primordial follicles, also described as the primordial follicle formation, demands for individual oocytes to associate with developing pre-granulosa cells, in a complex process that involves the breakdown of oocyte nests, oocyte separation, and subsequent recruitment of somatic (i.e. pre-granulosa) cells, which are regulated by circulating hormones and factors produced by the oocyte and somatic cells [28]. Newly created primordial follicles give rise to primary follicles that, in a series of transitions coordinated by gonadotropins, steroids, and other intraovarian factors, transform into preantral and then antral follicles and finally preovulatory follicles [6]. Among the signaling pathways that are important for primordial follicle assembly, apoptosis and autophagy are crucial in determining cellular fate. After nest separation, a large number of germ cells are lost by apoptosis; the mechanisms regulating cyst breakdown and germ cell death are still unclear. Indeed, much attention has been focused on germ cell elimination by apoptosis and the role of Bcl-2 gene family in regulating the balance between survival and death of oocytes before the formation of primordial follicles [29]. To date, autophagy has also been proposed to contribute in the mechanisms of prenatal and neonatal oocyte demise [30]. Increasing evidences showed that, in the immediate hours of the postnatal life, many tissues and organs evidence up-regulation of autophagy pathways, possibly acting as an adaptive response of the newborn organism to nutritional stress associated with the deprivation of placental nutrients [31]. The balance between quiescence and activation of the primordial follicle reserve seems to depend on a number of molecules. The phosphatidylinositol 3-kinase (PI3K) pathway was proposed as a key pathway playing a crucial integrative role by bridging the action of multiple factors in the balance

http://dx.doi.org/10.5772/62138

between follicle growth suppression, activation, and the maintenance of healthy quiescence [32]. In mammalian ovaries, postnatal depletion of oocytes occurs also by atresia of follicles. Follicular atresia is directed by granulosa cell apoptosis and affects all stages of follicular development. Interestingly, recent evidence from studies on rats shows that autophagy of germinal cells is an alternative route to induce follicular atresia in the ovary [33]. This implies the importance of autophagy in cellular elimination within the ovary.

3. Primordial to primary follicle transition

The concept of primordial follicle activation refers to the process by which primordial follicles gradually exit the nongrowing follicle pool and enter the intermediate or primary follicle stage [23]. The clarification of the mechanisms that regulate primordial follicle activation is an important issue for the success of assisted reproduction [4]. Whereas the primordial follicle activation seems to depend mainly on signals originating in the ovary, pituitary and metabolism-related hormones are required for folliculogenesis to proceed past the primary or secondary stage [34]. In the ovary, the crosstalk between oocytes and somatic cells (i.e. granulosa or theca cells) occurs at an early stage of follicular development [4]. The activation of primordial follicles is associated with oocyte growth, and simultaneous differentiation of the adjacent pre-granulosa cells occurs. During the transition into primary follicles (showing a complete layer of cuboidal granulosa cells), pre-granulosa cells change into a cuboidal shape [23], and in the process, they form an intermediate form of follicles presenting both cuboidal and flattened pre-granulosa cells [35]. The proliferation of granulosa cells allows to originate multiple layers of cells, and follicle develops to secondary, antral, and further advanced follicle stages [23,36,37].

Recent research revealed that factors secreted by the oocytes regulate the initiation of primordial follicle growth [38] (Figure 2). The tyrosine kinase receptor Kit (c-Kit) and two different isoforms of its ligand (kit ligand, KL), localized in oocytes and granulosa cells, stimulate oocyte growth and maintain it in meiotic arrest depending on FSHR levels. The up-regulated expression of KL, triggered by low concentrations of FSH, promotes a reduction in the ratio of KL/c-Kit and stimulates oocyte growth, whereas high concentrations of FSH enhance follicle development but impair oocyte growth [5]. Other important regulators of follicle growth are activin [39] and oocyte-derived growth differentiation factor-9 (GDF-9) [14,40]. GDF-9 promotes follicular survival and growth during transition due to suppression of granulosa cell apoptosis and follicular atresia, whereas activin promotes FSH release, antral cavity formation, and granulosa cell proliferation. Bone morphogenetic protein-15 (BMP15) has been shown to promote granulosa cell growth by stimulation of the proliferation of undifferentiated granulosa cells in an FSH-independent manner. It was shown that two markers of proliferation, Ki-67 and proliferating cell nuclear antigen (PCNA), are regulated by these oocyte-derived factors (for review, see Ref. [41]). It was also suggested that PCNA could act as a key regulator of the development of ovarian follicles. The time expression of PCNA in oocytes is coincident with the initiation of primordial follicle formation. By promoting the apoptosis of oocytes, PCNA can also regulate primordial follicle assembly in neonatal mouse ovaries [42]. Moreover,

proliferation of granulosa cells is increased by insulin-like growth factor-I (IGF-I), which was also associated with the regulation of follicular growth from the primordial stage [43]. The anti-Müllerian hormone (AMH) is synthesized early in the follicle formation, by the cuboidal granulosa cells of primordial follicles. This factor, subsequently produced by preantral and antral follicles, inhibits the initial recruitment of primordial ones as well as their further FSH-dependent growth [44].

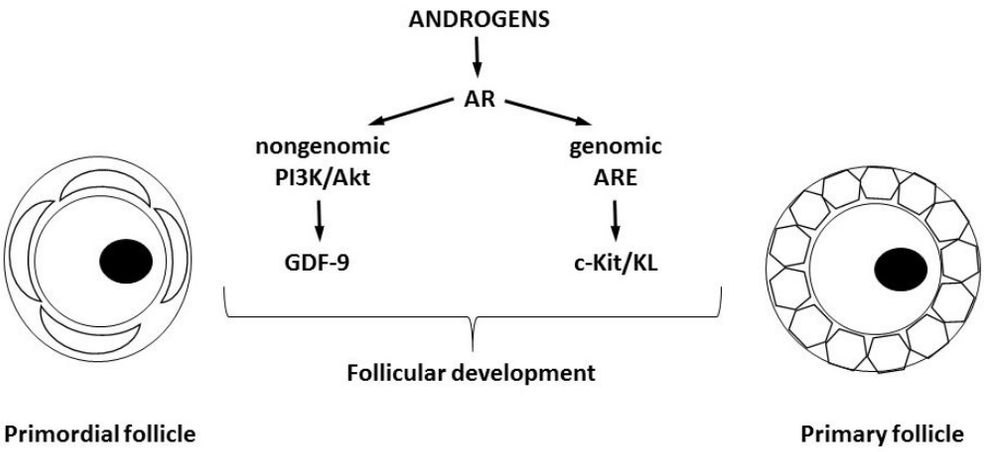


Figure 2. Mechanisms of androgen actions in follicular development. Physiological functions of androgens during primordial follicle recruitment are mediated through androgen response element (ARE)-dependent genomic actions and/or via PI3K/Akt nongenomic signaling pathway.GDF-9, growth differentiation factor-9; AR, androgen receptor.

The idea that androgens might regulate follicular development initially started with studies indicating AR expression in the different compartments of follicles throughout most stages of folliculogenesis [45–48]. However, AR expression pattern may differ between cell types, and in most species, AR is abundant in the preantral/antral stages of follicular development but declines as a follicle matures to the preovulatory stage [49–51]. Based on these observations, it was suggested that androgens might differentially regulate various stages of follicular development through an autocrine and/or paracrine way. It is generally accepted that androgens primarily affect preantral follicles and that their activities are important for preantral follicle growth and prevention of follicular atresia. Moreover, it seems possible that androgens are involved in the activation of primordial follicles [52–54] (Figure 2). How androgens influence primordial follicle recruitment and whether this is a primary or secondary response to androgens are still open-ended questions needing further investigation.

The mechanisms of primordial follicle activation can be studied using *in vitro* culture methods. However, until now, the success of primordial follicles culture as a method of oocyte growth has been limited to mice. Eppig and O'Brien [55] were the first to obtain mouse offspring derived from oocytes acquired from cultured primordial follicles. As to other species, several studies carried out in farm animals and primates showed that the transition of primordial into primary follicles in culture of cortical strips from caprine [56], bovine [57], baboon [58], and human [59] ovaries is possible. A confirmation of the normality of follicle development *in vitro* was obtained through the changes in follicle morphology and cell number as well as from

http://dx.doi.org/10.5772/62138

the stage-specific follicular responsiveness to above-mentioned factors or the development of steroidogenic capacity.

4. Primordial and primary follicle isolation

In vitro follicle growth is a promising fertility preservation strategy [19,60] despite that, in some mammalian species, including humans and pigs, the success has been limited when the process started with primordial follicles. This could be explained by the fact that adequate isolation methods and culture strategies have not yet been fully established, thereby impairing the ability to obtain mature gametes from the culture of isolated primordial follicles in those species. The manipulation of primordial follicles is a challenge due to their small size and the existing physical connections between the oocyte and the surrounding squamous granulosa cells, which are also poorly studied. Conversely, the conditions that support their activation and growth are not well defined. Several studies have indicated that primordial and primary follicles rapidly degenerate in cultures carried under multiple conditions [61–63]. For example, primordial follicles isolated from human ovarian tissue using collagenase digestion and subsequently cultured in collagen gels resulted in the degeneration of the follicles within 24 hours [64].

The species and the reproductive age of the ovarian tissue affect preantral follicle yield in the ovary because of the existence of a larger number of follicles and the easiness of the isolation method in neonatal and prepubertal ovaries compared with mature ovaries [65,66]. The success of either culture or transplantation of isolated follicles depends on the high quality of retrieved follicles. That is why an effective method for retrieving viable, preantral follicles is an essential condition. Different methods are currently available to isolate follicles for preantral follicle culture. The mechanical isolation methods include the use of fine-gauge needles or forceps to isolate follicles from mice [67], rats [68], pigs [69], cattle [70], and humans [64]; the combination of ovarian dissociation methods, such as grating or mincing, with sieving [71]; and the follicular dissection from the ovarian cortex using a skin-grafting knife and/or small scalpel blades [34,70]. The mechanical isolation methods have a main advantage, as they allow retrieving intact follicles, surrounded by the basement membrane and theca layers, although they are slow and laborious techniques that typically yield only a small number of follicles [72]. These technical problems can be avoided by the use of enzymes to aid follicle recovery. The incubation of ovarian tissue in collagenase and/or DNAse (e.g. Refs. [55,73]) softens and disaggregates the tissue matrix and allow detaching follicles from the surrounding stroma with the aid of needles. However, the degradation of the basement membrane and the absence of theca cell layers are the most common undesirable consequences of the use of enzymes in follicle isolation [74], as they foster the spontaneous loss of granulosa cells from the follicles in culture. Nevertheless, the time of enzyme exposure can be controlled to minimize the damage [75].

The ovarian stroma is dense and fibrous; thereby, it is more efficiently isolated using a combination of mechanical and enzymatic procedures that have been shown to preserve follicle viability [64,76–78]. Dolmans et al. [77] developed a new isolation protocol using Liberase Blendzyme 3. This blend of purified enzymes allowed the isolation of a high number

of preantral follicles, which were viable as well as morphologically and ultrastructurally normal. However, this type of Liberase is no longer produced. Therefore, the second generation of Liberase DH (Dispase High) Research Grade has been successfully tested for the preparation of human ovarian follicles [79]. However, the efficiency of the mixture may vary with the species. Our group recommends the use of Liberase TH (Thermolysin High) Research Grade to obtain a high number of fully isolated primordial follicles from porcine ovarian cortex [80], as it presents a really fibrous tissue. Using prepubertal gilt ovaries, we applied different types of Liberase (DH, TM, and TH) Research Grade and treatment protocols to isolate primordial follicles (Figure 3). The quality of the isolated follicles was evaluated by their general morphology and viability upon routine hematoxylin and eosin (H&E) and fluorescent staining, whereas their ultrastructure was assessed by electron microscopy. Additionally, to determine the purity of isolated follicles, a germ cell-specific protein, MSY2, was used to recognize oocytes. Liberase TH Research Grade was the mixture presenting a very high proportion of retrieved viable follicles whose majority exhibited good morphology with a complete granulosa cell layer. In addition, primordial follicles stained with either Hoechst 33342 or H&E indicated that Liberase TH Research Grade only occasionally induced atresia. This was supported by ultrastructural studies revealing that the oolemma-follicular cell interface was well preserved, which would allow the complex to express the correct metabolic profile (Figure 4). The results obtained in those experiments also showed that almost all of the Liberase TH Research Grade-isolated primordial follicles were MSY2 positive. As shown in the literature [81,82], primordial and primary follicles may rapidly degenerate after isolation because of the loss of critical connections between the oocyte and the granulosa cells. It seems that Liberase is a promising alternative to collagenase treatment, allowing the use of isolated primordial follicles for further reproductive studies.

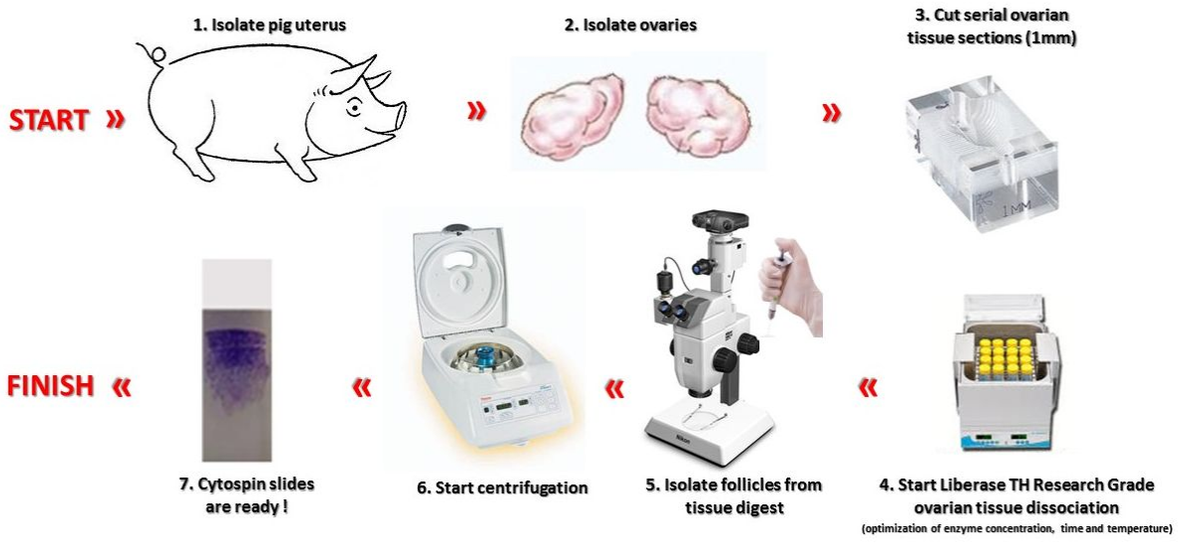


Figure 3. Pig ovarian medulla collection and preantral follicle isolation protocol. 1–3: Ovarian medulla collection (4- to 5-month-old prepubertal gilts); 4–6: isolation of primordial and primary follicles using different types of collagenase (types I, II, and IV) and Liberase (DH, TM, and TH); 7: evaluation of preantral follicles morphology (H&E staining). For details, see Ref. [80].

http://dx.doi.org/10.5772/62138

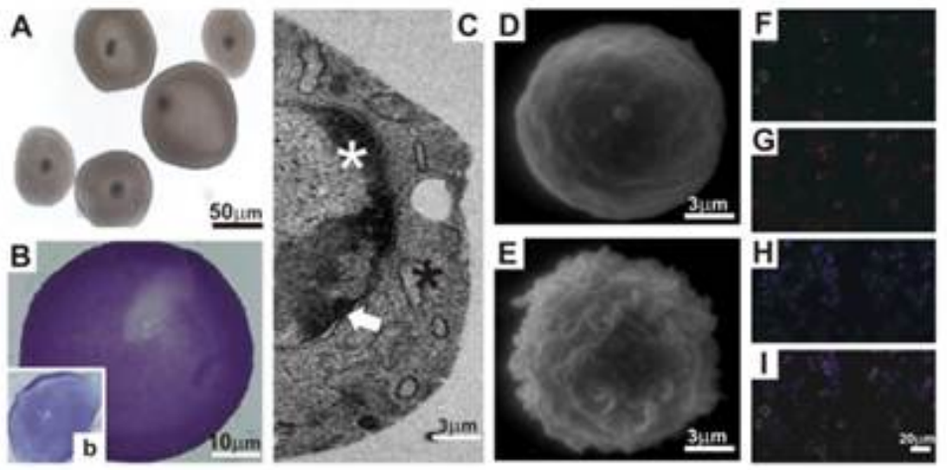


Figure 4. Morphology and ultrastructure of primordial and primary pig follicles isolated from ovarian medulla using Liberase TH-Research Grade. (A) Morphology of Liberase TH-treated pre-antral follicles (light microscopy), (B) morphology of Liberase TH-isolated pre-antral follicles stained with hematoxylin and eosin; (b) interrupted granulosa cells layer in pre-antral pig follicles isolated with collagenase (type II); (C) Transmission electron microscopy (TEM) showed a single uninterrupted layer of cuboid follicular cells (black asterisk) surrounding the oocyte (white asterisk), which was bordered by a continuous basal lamina (arrow). Scanning electron microscopy (SEM) of primordial (D) and primary (E) follicles isolated using Liberase TH. A continuous layer of cuboid follicular cells surrounds the oocyte in the primary follicle (E) while in the primordial follicle a flattened layer of cells covers the oocyte; immunoconfocal images recorded from three selected areas of centrifuged ovarian digest: follicles were stained for actin (F), MS2Y (G) and with DAPI (H), merged images (I).

5. Primordial and primary follicle culture

The clinical application of IVFG is still at the investigational stage, in a laboratory setting, although it stands a robust approach to study the basic biology of the ovary or the follicle under a controlled yet adjustable environment. Multiple culture systems have been developed to support the development of isolated preantral follicles [69,72,83], each one with its own advantages and providing useful insights into the follicle physiology. By this time, hydrogel-based follicle culture systems have been well characterized. The oocyte and the surrounding granulosa cells interact with each other and the environment, maintaining the same spatial location, connections, and dimensionality as in the intact ovary. The *in vitro* growth and development of mouse preantral follicles was successfully supported by alginate-based hydrogels, a substrate that was also applied to several large mammalian species, including dogs [84], rhesus monkeys [19], and humans [85], resulting in stage IV oocytes (human) [86], meiosis II (MII)-arrested eggs, and fertilized two-cell embryos (rhesus macaque) [87]. This developmental stage has not been reached in other systems.

It is commonly agreed that early follicular growth is largely independent of a gonadotropin stimulus; instead, it seems that it is controlled by paracrine and autocrine signals originating from several sources in the ovary, including stromal cells, macrophages, and other follicles [38]. Recent studies showed that these local factors may also play an important role in *in*

vitro culture, supporting the growth of isolated preantral follicles: isolated primary ovarian follicles survived and grew when cocultured with purified ovarian stroma including theca-interstitial cells and macrophages [88] or with mouse embryonic fibroblasts (MEFs) as a feeder cell layer [89]. Coculture with MEFs resulted in an increased follicle survival, growth, and differentiation until antral follicles contained meiotically competent oocytes capable of reaching metaphase II in response to adequate hormone stimulation [89]. As suggested in those studies, individual primary follicles require factors beyond the standard culture media additives, including insulin, transferrin, selenium, fetuin, bovine serum albumin, and FSH [90], which can be supplied *in vitro* by coculture with stromal cells or MEFs; nevertheless, *in vivo* observations also suggest that follicles themselves may have a stimulatory effect on IVFG, because, in the mammalian ovarian cortex, the distinctive architecture and follicle distribution may influence follicle development: primordial and primary follicles are located close to the rigid, collagen-dense cortical stroma, whereas larger, growing follicles are typically closer to the interior medulla, which presents a less rigid stroma [91]. It has been shown, in a study examining the spatial relationship of follicles within ovaries, that follicles surrounded by growing follicles are more likely to be growing, suggesting the existence of an *in vivo* stimulatory effect of other follicles [92] that could be exerted by signals originating from both the oocyte and the growing follicles, which enhance the differentiation of preantral follicles.

6. Summary

In summary, the ability to sustain preantral follicle growth *in vitro* while supporting the acquisition of oocyte competence is of great scientific interest. This relies on supplying oocytes for assisted reproductive technologies and broadening our understanding of somatic cell/oocyte interactions in species characterizing by prolonged follicular growth, such as humans and pigs. IVGF is becoming a useful tool to assess follicular development, offering also the potential to preserve reproductive options in cases of polycystic ovarian syndrome (PCOS), premature ovarian failure, or definitive sterility (post-oncotherapy). In addition, it is known that certain ovarian dysfunctions, such as PCOS and gonadotropin poor responsiveness, are consequences of deregulated follicle growth at this transitional stage. Therefore, the elucidation of molecular and cellular mechanisms involved in the control of follicular development during transition from preantral to early antral stage may provide an important insight into the pathophysiology and rational treatment of these disorders.

Acknowledgements

This work was supported by DEC-2013/09/B/NZ9/00226 from the National Polish Science Centre.

Author details

Duda Malgorzata[1*], Grzesiak Malgorzata[2], Knet-Seweryn Malgorzata[1] and Zbigniew Tabarowski[3]

*Address all correspondence to: duda.m@op.pl

1 Department of Endocrinology, Institute of Zoology, Jagiellonian University, Kraków, Poland

2 Department of Animal Physiology and Endocrinology, University of Agriculture, Kraków, Poland

3 Department of Experimental Hematology, Institute of Zoology, Jagiellonian University, Kraków, Poland

Conflict of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Author's contributions

Malgorzata Duda and Malgorzata Grzesiak prepared the paper, Malgorzata Knet-Seweryn collected articles for this chapter, and Tabarowski Zbigniew reviewed the paper. All authors read and approved the final manuscript version.

References

[1] McGee EA, Hsueh AJW. Initial and cyclic recruitment of ovarian follicles. Endocrine Reviews. 2000;21:200–214.

[2] Monniaux D, Clement F, Dalbies-Tran R, Estienne A, Fabre S, Mansanet C, Monget P. The ovarian reserve of primordial follicles and the dynamic reserve of antral growing follicles: what is the link? Biology of Reproduction. 2014;90:1–11.

[3] Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. Endocrine Reviews. 1996;17:121–155.

[4] Matzuk MM, Burns KH, Viveiros MM, Eppig JJ. Intercellular communication in the mammalian ovary: oocytes carry the conversation. Science. 2002;296:2178–2180. DOI: 10.1126/science.1071965

[5] Fortune JE, Ribera GM, Yang MY. Follicular development: the role of follicular microenvironment in selection of dominant follicle. Animal Reproduction Science. 2004;82:109–126.

[6] Drummond AE. The role of steroids in follicular growth. Reproductive Biology and Endocrinology. 2006;4:16.

[7] Huynh K, Jones G, Thouas G, Britt KL, Simpson ER, Jones MEE. Estrogen is not directly required for oocyte developmental competence. Biology of Reproduction. 2004;70:1263–1269.

[8] Stouffer RL. Progesterone as a mediator of gonadotropin action in the corpus luteum: beyond steroidogenesis. Human Reproduction Update. 2003;9:99–117.

[9] Hild-Petito S, West NB, Brenner RM, Stouffer RL. Localization of androgen receptor in the follicle and corpus luteum of the primate ovary during the menstrual cycle. Biology of Reproduction. 1991;44:561–568.

[10] Weil SJ, Vendola K, Zhou J, Adesanya OO, Wang J, Okafor J, Bondy CA. Androgen receptor gene expression in the primate ovary: cellular localization, regulation, and functional correlations. Journal of Clinical Endocrinology and Metabolism. 1998;83:2479–2485.

[11] Vendola K, Zhou J, Wang J, Bondy CA. Androgens promote insulin-like growth factor-I and insulin-like growth factor-I receptor gene expression in the primate ovary. Human Reproduction. 1999;14:2328–2332.

[12] Vendola K, Zhou J, Wang J, Famuyiwa OA, Bievre M, Bondy CA. Androgens promote oocyte insulin-like growth factor I expression and initiation of follicle development in the primate ovary. Biology of Reproduction. 1999;61:353–357.

[13] Lenie S, Smitz J. Functional AR signaling is evident in an *in vitro* mouse follicle culture bioassay that encompasses most stages of folliculogenesis. Biology of Reproduction. 2009;80:685–695.

[14] Skinner MK. Regulation of primordial follicle assembly and development. Human Reproduction Update. 2005;11:461–471.

[15] Tingen CM, Bristol-Gould SK, Kiesewetter SE, Wellington JT, Shea L, Woodruff TK. Prepubertal primordial follicle loss in mice is not due to classical apoptotic pathways. Biology of Reproduction. 2009;81:16–25.

[16] Vanderstichele H, Delaey B, de Winter J, de Jong F, Rombauts L, Verhoeven G, Dello C, van de Voorde A, Briers T. Secretion of steroids, growth factors and cytokines by immortalized mouse granulosa cells lines. Biology of Reproduction. 1994;50:1190–1202.

[17] Picton HM, Muruvi W, Jin P. Interaction of oocyte and somatic cells. In: Tan SL, Chian RC, Buckett WM, editors. In-Vitro Maturation of Human Oocytes—Basic Science to Clinical Application. Oxon, UK: Informa Health; 2007. p. 37–48.

[18] Jeruss JS, Woodruff TK. Preservation of fertility in patients with cancer. New England Journal of Medicine. 2009;360:902–911.

[19] Xu M, West-Farrell ER, Stouffer RL, Shea LD, Woodruff TK, Zelinski MB. Encapsulated three-dimensional culture supports development of nonhuman primate secondary follicles. Biology of Reproduction. 2009;81:587–594.

[20] Schmidt KT, Larsen EC, Andersen CY, Andersen AN. Risk of ovarian failure and fertility preserving methods in girls and adolescents with a malignant disease. BJOG: An International Journal of Obstetrics and Gynaecology. 2010;117:163–174.

[21] McNatty KP, Fidler AE, Juengel JL, Quirke LD, Smith PR, Heath DA, Lundy T, O'Connell A, Tisdall DJ. Growth and paracrine factors regulating follicular formation and cellular function. Molecular and Cellular Endocrinology. 2000;163:11–20.

[22] Hyttel P, Sinowatz F, Vejlsted M, Betteridge K. Essentials of Domestic Animal Embryology. 1st ed. Edinburgh: Elsevier; 2010.

[23] Picton HM. Activation of follicle development: the primordial follicle. Theriogenology. 2001;55:1193–1210.

[24] Young JM, McNeilly AS. Theca: the forgotten cell of the ovarian follicle. Reproduction. 2010;140:489–504.

[25] Binelli M, Murphy BD. Coordinated regulation of follicle development by germ and somatic cells. Reproduction, Fertility and Development. 2010;22:1–12.

[26] McLaughlin EA, McIver SC. Awakening the oocyte: controlling primordial follicle development. Reproduction. 2009;137:1–11.

[27] van den Hurk R, Zhao J. Formation of mammalian oocytes and their growth, differentiation and maturation within ovarian follicles. Theriogenology. 2005;63:1717–1751.

[28] Pepling ME. Follicular assembly: mechanisms of action. Reproduction. 2012;143:139–149.

[29] Fulton N, Martins da Silva SJ, Bayne RA, Anderson RA. Germ cell proliferation and apoptosis in the developing human ovary. The Journal of Clinical Endocrinology & Metabolism. 2005;90:4664–4670.

[30] Rodrigues P, Limback D, McGinnis LK, Plancha CE, Albertini DF. Multiple mechanisms of germ cell loss in the perinatal mouse ovary. Reproduction. 2009;137:709–720.

[31] Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhisa T, Mizushima M. The role of autophagy during the early neonatal starvation period. Nature. 2004;432:1032–1036.

[32] Kerr JB, Myers M, Anderson RA. The dynamics of the primordial follicle reserve. Reproduction. 2013;146:205–215.

[33] Escobar ML, Echeverría OM, Ortíz R, Vázquez-Nin GH. Combined apoptosis and autophagy, the process that eliminates the oocytes of atretic follicles in immature rats. Apoptosis. 2008;13:1253–1266.

[34] Picton HM, Harris SE, Muruvi W, Chambers EL. The *in vitro* growth and maturation of follicles. Reproduction. 2008;136:703–15.

[35] Da Silva-Buttkus P, Jayasooriya GS, Mora JM, Mobberley M, Ryder TA, Baithun M, Stark J, Frank S, Hardy K. Effect of cell shape and packing density on granulosa cell proliferation and formation of multiple layers during early follicle development in the ovary. Journal of Cell Science. 2008;121:3890–3900.

[36] Lintern-Moore S, Moore GP. The initiation of follicle and oocyte growth in the mouse ovary. Biology of Reproduction. 1979;20:773–778.

[37] Hirshfield AN. Theca cells may be present at the outset of follicular growth. Biology of Reproduction. 1991;44:1157–1162.

[38] Peters H, Byskov AG, Himelstein-Braw R, Faber M. Follicular growth: the basic event in the mouse and human ovary. Journal of Reproduction and Fertility. 1975;45:559–566.

[39] Knox RV. Recruitment and selection of ovarian follicle for determination of ovulation rate in pig. Domestic Animal Endocrinology. 2005;29:385–397.

[40] McGrath SA, Esquela AF, Lee SJ. Oocyte-specific expression of growth differentiation factor-9. Molecular Endocrinology. 1995;9:131–136.

[41] Hsueh AJ, Kawamura K, Cheng Y, Fauser BC. Intraovarian control of early folliculogenesis. Endocrine Reviews. 2015;36:1–24.

[42] Xu B, Hua J, Zhang Y, Jiang X, Zhang H, Ma T, Zheng W, Sun R, Shen W, Sha J, Cooke HJ, Shin Q. Proliferating cell nuclear antigen (PCNA) regulates primordial follicle assembly by promoting apoptosis of oocytes in fetal and neonatal mouse ovaries. PLoS One. 2011;6:e16046.

[43] Qu J, Godin PA, Nisolle M, Donnez J. Expression of receptors for insulin-like growth factor-I and transforming growth factor-beta in human follicles. Molecular Human Reproduction. 2000;6:137–145.

[44] Visser JA, de Jong FH, Laven JSE, Themmen APN. Anti-Müllerian hormone: a new marker for ovarian function. Reproduction. 2006;131:1–9.

[45] Hillier SG, Tetsuka M, Fraser HM. Location and developmental regulation of androgen receptor in primate ovary. Human Reproduction. 1997;12:107–111.

[46] Szoltys M, Slomczynska M. Changes in distribution of androgen receptor during maturation of rat ovarian follicles. Experimental and Clinical Endocrinology & Diabetes. 2000;108:228–234.

[47] Slomczynska M, Tabarowski Z. Localization of androgen receptor and cytochrome P450 aromatase in the follicle and corpus luteum of the porcine ovary. Animal Reproduction Science. 2001;65:127–134.

[48] Juengel JL, Heath DA, Quirke LD, McNatty KP. Oestrogen receptor alpha and beta, androgen receptor and progesterone receptor mRNA and protein localization within the developing ovary and in small growing follicles of sheep. Reproduction. 2006;131:81–92.

[49] Cardenas H, Herrick JR, Pope WF. Increased ovulation rate in gilts treated with dihydrotestosterone. Reproduction. 2002;123:527–533.

[50] Hampton JH, Manikkam M, Lubahn DB, Smith MF, Garveric HA. Androgen receptor mRNA expression in the bovine ovary. Domestic Animal Endocrinology. 2004;27:81–88.

[51] Rice S, Ojha K, Whitehead S, Mason H. Stage-specific expression of androgen receptor, follicle stimulating hormone receptor, and anti-Mullerian hormone type II receptor in single, isolated human preantral follicles: relevance to polycystic ovaries. The Journal of Clinical Endocrinology and Metabolism. 2007;92:1034–1040.

[52] Abbott DH, Dumesic DA, Levine JE, Dunaif A, Padmanabhan V. Animal models and fetal programming of PCOS. In: Azziz R, Nestler JE, Dewailly D, editors. Contemporary Endocrinology: Androgen Excess Disorders in Women: Polycystic Ovary Syndrome and Other Disorders. 2nd ed. Humana Press Incorporation, Totowa, NY; 2006. p. 259–272.

[53] Smith P, Stickler TL, Veiga-Lopez A, Padmandabhan V. Developmental programming: differential effects of prenatal testosterone and dihydrotestosterone on follicular recruitment, depletion of follicular reserve and ovarian morphology in sheep. Biology of Reproduction. 2009;80:726–736.

[54] Magamage MPS, Zengyo M, Moniruzzaman M, Miyano T. Testosterone induces activation of porcine primordial follicles *in vitro*. Reproductive Medicine and Biology. 2011;10:21–30.

[55] Eppig JJ, O'Brien MJ. Development *in vitro* of mouse oocytes from primordial follicles. Biology of Reproduction. 1996;54:197–207.

[56] Silva JR, van den Hurk R, de Matos MH, dos Santos RR, Pessoa C, de Moraes MO, de Figueiredo. Influences of FSH and EGF on primordial follicles during *in vitro* culture of caprine ovarian cortical tissue. Theriogenology. 2004;61:1691–1704.

[57] Wandji SA, Srsen V, Voss AK, Eppig JJ, Fortune JE. Initiation *in vitro* of growth of bovine primordial follicles. Biology of Reproduction. 1996;55:942–948.

[58] Fortune JE, Kito S, Wandji SA, Srsen V. Activation of bovine and baboon primordial follicles *in vitro*. Theriogenology. 1998;49:441–449.

[59] Hovatta O, Silye R, Abir R, Krausz T, Winston RM. Extracellular matrix improves survival of both stored and fresh human primordial and primary ovarian follicles in long-term culture. Human Reproduction. 1997;12:1032–1036.

[60] Xu M, Barrett SL, West-Farrell ER, Kondapalli LA, Kiesewetter SE, Shea LD, Woodruff TK. *In vitro* grown human ovarian follicles from cancer patients support oocyte growth. Human Reproduction. 2009;24:2531–2540.

[61] Abir R, Fisch B, Nitke S, Okon E, Raz A, Ben Rafael Z. Morphological study of fully and partially isolated early human follicles. Fertility and Sterility. 2001;75:141–146.

[62] Hovatta O, Wright C, Krausz T, Hardy K, Winston RM. Human primordial, primary and secondary ovarian follicles in long-term culture: effect of partial isolation. Human Reproduction. 1999;14:2519–2524.

[63] O'Brien MJ, Pendola JK, Eppig JJ. A revised protocol for *in vitro* development of mouse oocytes from primordial follicles dramatically improves their developmental competence. Biology of Reproduction. 2003;68:1682–1686.

[64] Abir R, Roizman P, Fisch B, Nitke S, Okon E, Orvieto R, Ben Rafael Z. Pilot study of isolated early human follicles cultured in collagen gels for 24 hours. Human Reproduction. 1999;14:1299–1301.

[65] Figueiredo JR, Hulshof SC, Van den Hurk R, Ectors FJ, Fontes RS, Nusgens B, Bevers MM, Beckers JF. Development of a new mechanical method for the isolation of intact preantral follicles from fetal, calf and adult bovine ovaries. Theriogenology. 1993;40:789–799.

[66] Carambula SF, Goncalves PB, Costa LF, Figueiredo JR, Wheeler MB, Neves JP, Mondadori RG. Effect of fetal age and method of recovery on isolation of preantral follicles from bovine ovaries. Theriogenology.1999;52:563–571.

[67] Cortvrindt R, Smitz J, Van Steirteghem AC. *In vitro* maturation, fertilization and embryo development of immature oocytes from early preantral follicles from prepubertal mice in a simplified culture system. Human Reproduction. 1996;11:2656–2666.

[68] Zhao J, Dorland M, Taverne MA, Van Der Weijden GC, Bevers MM, Van Den Hurk R. *In vitro* culture of rat pre-antral follicles with emphasis on follicular interactions. Molecular Reproduction and Development. 2000;55:65–74.

[69] Wu J, Emery BR, Carrell DT. *In vitro* growth, maturation, fertilization, and embryonic development of oocytes from porcine preantral follicles. Biology of Reproduction. 2001;64:375–381.

[70] Gutierrez CG, Ralph JH, Telfer EE, Wilmut I, Webb R. Growth and antrum formation of bovine preantral follicles in long-term culture *in vitro*. Biology of Reproduction. 2000;62:1322–1328.

[71] Jewgenow K. Role of media, protein and energy supplements on maintenance of morphology and DNA-synthesis of small preantral domestic cat follicles during short-term culture. Theriogenology. 1998;49:1567–1577.

[72] Telfer EE, Binnie JP, McCaffery FH, Campbell BK. *In vitro* development of oocytes from porcine and bovine primary follicles. Molecular and Cellular Endocrinology. 2000;163:117–123.

[73] Newton H, Picton HM, Gosden RG. *In vitro* growth of oocyte-granulosa cell complexes isolated from cryopreserved ovine tissue. Journal of Reproduction and Fertility. 1999;115:141–150.

[74] Nayudu PL, Fehrenbach A, Kiesel P, Vitt UA, Pancharatna K, Osborn SM. Progress towards understanding follicle development *in vitro*: appearances are not deceiving. Archives of Medical Research. 2001;32:587–594.

[75] Shuttleworth G, Broughton Pipkin F, Hunter MG. *In vitro* development of pig preantral follicles cultured in a serum-free medium and the effect of angiotensin II. Reproduction. 2002;123:807–818.

[76] Oktay K, Nugent D, Newton H, Salha O, Chatterjee P, Gosden RG. Isolation and characterization of primordial follicles from fresh and cryopreserved human ovarian tissue. Fertility and Sterility. 1997;67:481–486.

[77] Dolmans MM, Michaux N, Camboni A, Martinez-Madrid B, Van Langendonckt A, Nottola SA, Donnez J. Evaluation of Liberase, a purified enzyme blend, for the isolation of human primordial and primary ovarian follicles. Human Reproduction. 2006;21:413–420.

[78] Rice S, Ojha K, Mason H. Human ovarian biopsies as a source of pre-antral follicles. Human Reproduction. 2007;23:600–605.

[79] Kristensen SG, Rasmussen A, Byskov AG, Andersen CY. Isolation of pre-antral follicles from human ovarian medulla tissue. Human Reproduction. 2011;26:157–166.

[80] Duda M, Grzesiak M, Tabarowski Z, Tomanek M. Isolation of primordial and primary follicles from porcine ovarian medulla tissue. In: Reproduction Abstracts Vol. 1, World Congress of Reproductive Biology 2014; 2–4 September 2014, Edinburgh, UK. p. 142. Available from: http://www.wcrb2014.org/Portals/0/Conferences/Abstracts/WCRB_2014/WCRB_2014P142.pdf.

[81] Romero S, Smitz J. Exposing cultured mouse ovarian follicles under increased gonadotropin tonus to aromatizable androgens influences the steroid balance and reduces oocyte meiotic capacity. Endocrine. 2010;38:243–253.

[82] Hornick JE, Duncan FE, Shea LD, Woodruff TK. Isolated primate primordial follicles require a rigid physical environment to survive and grow *in vitro*. Human Reproduction. 2012;27:1801–1810.

[83] Abir R, Nitke S, Ben-Haroush A, Fisch B. *In vitro* maturation of human primordial ovarian follicles: clinical significance, progress in mammals, and methods for growth evaluation. Histology and Histopathology. 2006;21:887–898.

[84] Songsasen N, Woodruff TK, Wildt DE. *In vitro* growth and steroidogenesis of dog follicles are influenced by the physical and hormonal microenvironment. Reproduction. 2011;142:113–122.

[85] Smitz J, Dolmans MM, Donnez J, Fortune JE, Hovatta O, Jewgenow K, Picton HM, Plancha C, Shea LD, Stouffer RL, Telfer EE, Woodruff TK, Zelinski MB. Current achievements and future research directions in ovarian tissue culture, *in vitro* follicle development and transplantation: implications for fertility preservation. Human Reproduction Update. 2010;16:395–414.

[86] Kreeger PK, Deck JW, Woodruff TK, Shea LD. The *in vitro* regulation of ovarian follicle development using alginate-extracellular matrix gels. Biomaterials. 2006;27:714–723.

[87] Ting AY, Yeoman RR, Lawson MS, Zelinski MB. *In vitro* development of secondary follicles from cryopreserved rhesus macaque ovarian tissue after slow-rate freeze or vitrification. Human Reproduction. 2011;26:2461–2472.

[88] Tingen CM, Kiesewetter SE, Jozefik J, Thomas C, Tagler D, Shea L, Woodruff TK. A macrophage and theca cell-enriched stromal cell population influences growth and survival of immature murine follicles *in vitro*. Reproduction. 2011;141:809–820.

[89] Tagler D, Tu T, Smith RM, Anderson NR, Tingen CM, Woodruff TK, Shea LD. Embryonic fibroblasts enable the culture of primary ovarian follicles within alginate hydrogels. Tissue Engineering. Part A. 2012;18:1229–1238.

[90] Xu M, Kreeger PK, Shea LD, Woodruff TK. Tissue-engineered follicles produce live, fertile offspring. Tissue Engineering. 2006;12:2739–2746.

[91] Hornick, JFE, Duncan L, Shea LD, Woodruff TK. Multiple follicle culture supports primary follicle growth through paracrine-acting signals. Reproduction. 2013;145:19–32.

[92] Da Silva-Buttkus P, Marcelli G, Franks S, Stark J, Hardy K. Inferring biological mechanisms from spatial analysis: prediction of a local inhibitor in the ovary. Proceedings of the National Academy of the United States of America. 2009;106:456–461.