Early Follicles

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Early follicles' development, especially the activation of primordial follicles, is strictly modulated by a network of signaling pathways. Recent advance in ovarian physiology has been allowed the development of several therapies to improve reproductive outcomes by manipulating early folliculogenesis. Among these, in vitro activation (IVA) has been recently developed to extend the possibility of achieving genetically related offspring for patients with premature ovarian insufficiency and ovarian dysfunction. This method was established based on basic science studies of the intraovarian signaling pathways: the phosphoinositide 3-kinase (PI3K)/Akt and the Hippo signaling pathways. These two pathways were found to play crucial roles in folliculogenesis from the primordial follicle to the early antral follicle. Following the results of rodent experiments, IVA was implemented in clinical practice. There have been multiple recorded live births and ongoing pregnancies. Further investigations are essential to confirm the efficacy and safety of IVA before used widely in clinics.

in vitro activation | hippo signaling pathway | PI3K/Akt/FOXO3 pathway

1. Introduction

The majority of achievements in medical science practice are based on basic scientific research. Improved understanding of reproductive physiology has also allowed remarkable advances in artificial reproductive technologies, giving a higher chance to achieve parenthood for millions of infertile couples [1].

As a consequence of global modernization, a higher number of advanced aged infertile women with diminished ovarian reserve (DOR) have been diagnosed in the last decades [2][3]. Due to innovations in oncological treatment, the number of cancer survivors at the reproductive age has been increasing, leading to a higher prevalence of premature ovarian insufficiency (POI) [4]. In addition, there is an increasing necessity for fertility preservation (FP).

During last decades, oocyte donation which cannot fulfill wishes of patients to give birth to genetically related offspring is often the option for patients with DOR and POI. To expand reproductive possibilities to DOR and POI patients, considerable efforts to investigate molecular mechanisms underlying folliculogenesis, leading to a variety of new approaches including follicle regeneration, rejuvenation, and activation [5].

Among these, the activation of early follicles (EFs) including primordial, primary, secondary, and early antral follicles has been recently achieved. For developing the efficient activation system, many basic studies using the animal-model were conducted to identify the signaling pathways governing the activation of early follicles.

IVA has been recently introduced and gradually implemented in clinical practice. This innovation was established from numerous animal experiments, including genetic manipulation studies illustrating the molecular mechanism of two involving signaling pathways in folliculogenesis. The first one is the PI3K/Akt/forkhead box O3 (FOXO3) pathway, which has a crucial role in the activation of primordial follicles (PFs) [6][7][8][9]. The other one is the Hippo signaling pathway which has been recently illustrated to modulate the progress of follicles from secondary to antral stage [10][11]. Based on results obtained from animal-model and in vitro experiments, IVA was implemented to treat POI and DOR women. Healthy babies and other encouraging outcomes have been reported from different groups [11][12][13][14][15][16][17]. Furthermore, several studies suggested the correlation between Hippo pathway's genes abnormality and polycystic ovary syndrome (PCOS) [18][19][20], leading to a possibility to ameliorate reproductive outcomes of PCOS patients by manipulating the Hippo signaling pathway [21].

2. The Activation of the EFs In Vitro

Folliculogenesis is a complicated process which is generally divided into two stages: the first one is early stage from the PFs to the early antral follicles stage, and the latter one is the early antral follicles to ovulatory stage (Figure 1) [22]. In mammalian ovaries, EFs are the largest population comprising of the PFs, primary follicles, secondary ones, and early antral follicles.

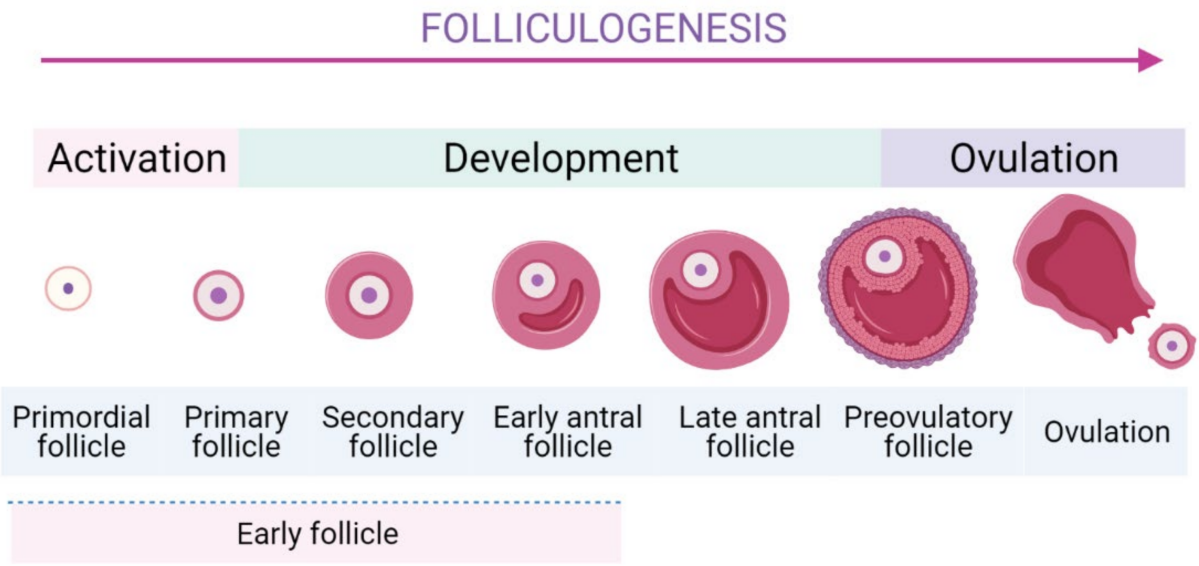

Figure 1. The schematic folliculogenesis from primordial to ovulatory stages.

To form the growing follicles, a small number of PFs are cyclically and periodically recruited in the growing pool. The most specific feature of PFs is their long-term dormancy and survival for several decades, ensuring the longevity for female reproductive possibility. Thus, they represent a finite source of ovarian follicles and their recruitment is an indispensable step as it determines reproductive lifespan [23][24]. The activation of EFs is modulated strictly to ensure only a small number of PFs can be activated [10][25]. Subsequently, they undergo continuous folliculogenesis to develop to later stages of development until the ovulation occurs. In PF, a dormant oocyte is enveloped by a single layer of flattened granulosa cells (GCs). In response to stimulators of PI3K/Akt

pathway, mTORC1 in GCs is activated [26] and then GCs start to proliferate, cuboidalize, and exclude SMADs proteins [26][27]. These dynamic changes are important signals to initiate the activation of PFs [27]. Moreover, several genes expressed in the GCs were identified to promote the PFs' activation [26][28]. The internal communication between oocyte and GCs is crucial during follicle development [26].

Although the number of PFs decreases gradually through apoptosis and recruitment, the pool of PFs is not completely exhausted even at the age of menopause [24]. Additionally, it is reported that three out of four POI patients have residual dormant PFs remaining in their ovaries [29]. PFs are the ideal population for PF as they could resist chemotherapies and are well-preserved during cryopreservation [30]. Therefore, consideration to develop an in vitro approach to control the activation of PFs has attracted extensive interest in the past decades [24]. Recently, a number of innovative approaches have been suggested to promote the EFs' development by using key agents to imitate the physiology ovarian environment.

The first in vitro experiment of PFs was conducted on mice by culturing the whole intact ovaries in serum-containing medium. The spontaneous activation of a small number of PFs in the medulla region, which is similar to the first wave of physiologic PFs activation, was noted. Additionally, the addition of epidermal growth factor (EGF) could enhance the follicle recovery [31]. In bovine and ovine species, culturing ovarian cortex with serum-free medium was also found to activate the PFs [32][33][34]. In human ovary, PFs activated spontaneously in the cultured ovarian cortex regardless of the presence of serum [35][36][37]. The two-step culture procedure using activin comprised of ovarian cortical strips culture in human followed by isolation and culture of the acquired secondary follicles was reported to yield antral stage follicles from the PFs. However, as the timing of follicle growth in this system is highly irregular, the finding has yet to be repeated [37].

These findings suggested that the PFs could be suppressed under the physiological environment, or there are in vitro factors stimulating the PF activation [9]. Although the number of activated PFs was limited in these studies, these encouraging findings placed the important foundation for the development of the activation of EFs in vitro controlled environment. Subsequently, accumulated understanding in mechanisms modulating the activation of EFs has been achieved, leading to a step closer to its potential clinical applicability. The activation of EFs is strictly governed by a number of signaling pathways and components as discussed in the following sections.

3. Summary

Numerous researches provided scientific evidence for pathways underlying the IVA approach. IVA offered encouraging outcomes to the poor prognostic infertile women in the clinic. Individualization in treatment is a crucial aspect of clinical practice. In patients with DOR or early stage of POI, drug-free IVA is more beneficial compared to conventional IVA as it decreases the invasiveness of surgical approach and avoids the unfavorable effects of tissue culture on follicles. In terms of FP, IVA should be applied to only patients with low ovarian reserve and the requirement for motherhood achievement is urgent.

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