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Does inositol ratio orchestrate the fate of ovarian follicles?

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A B S T R A C T

The ratio of myo-inositol (MI) to D-chiro-inositol (DCI) seems to regulate the steroidogenesis in recruited follicles. While MI favors the increase of estrogens, DCI contributes to an androgenic environment, either by stimulating the biosynthesis of testosterone or by reducing its conversion to estradiol. Based on available evidence, we put forward the hypothesis that the different MI:DCI ratios influence the selection process, determining the dominant follicle and the follicles that will undergo atresia. We also suggest that the MI:DCI ratio may be regulated by follicles' intrinsic characteristics, which possibly drive the cohort recruitment at each ovarian cycle.

Introduction

Human folliculogenesis is a complex and fascinating process of reproduction [1,2], though some of the mechanisms involved remain partially unexplained. At every reproductive cycle, a cohort of antral follicles in the ovaries is recruited for final maturation [3]. Generally, only a single one of them becomes dominant and reaches the preovulatory stage; the others undergo atresia. A widely accepted hypothesis to explain such selection involves the follicular responsiveness to the follicle–stimulating hormone (FSH), suggesting that the follicle with a greater number of granulosa cells or a higher density of FSH receptors will grow faster than the others [4]. However, as atretic follicles have their own specific role and provide the androgens that increase female sexual desire [5,6], a randomly driven selection process appears somehow far–fetched. Taking into account more recent findings, here we propose a leading role of inositol ratio in determining the follicles' fate. Such hypothesis would constitute a major step forward in the understanding of ovarian folliculogenesis, supporting the idea that the process is more sophisticated than just winning a race.

Myo-inositol and D-chiro-inositol in ovarian physiology: What do we know?

Fig. 1 schematically depicts the suggested roles of inositol isomers in the ovarian steroidogenesis. As second messengers of FSH in the granulosa cells, myo-inositol (MI) and its derivatives are key mediators in the selection of the dominant follicle [7]. The activity of the enzyme CYP19A1 (aromatase) increases under the stimulus of FSH [8]. Consequently, the recruited follicles start to produce estradiol, which in turn suppresses the release of gonadotropin-releasing hormone (GnRH) and FSH through a negative feedback on either the hypothalamus and the anterior pituitary [9]. Such regulatory cross-talk initiates the ping-pong mechanism that leads to the selection of the dominant follicle [10]. While undeniably MI has a key role in this process, the ovaries also contain D-chiro-inositol (DCI), whose activities have been long underestimated. The presence of a specific epimerase enzyme, which converts MI into DCI, indicates that the latter is also essential for ovarian physiology and that its concentration is tightly regulated. In fact, epimerase's activity is tissue specific [11], with different MI:DCI ratios in different tissues and organs. For example, MI:DCI ratio is 40:1 in the peripheral blood [12], while it becomes about 20:1 in the thecal cells [13] and within the range 70:1–100:1 in the follicular fluid [14] of dominant follicles. To the best of our knowledge, indications about the inositol ratio in the granulosa cells are still unavailable. Altered MI:DCI ratios are characteristic of pathological conditions such as hyperinsulinemia, which leads to increased MI-to-DCI conversion in the ovaries [15]. Insulin resistant PCOS women, indeed, exhibit decreased MI:DCI ratios both in thecal cells [13] and in the follicular fluid [14]. Moreover, studies on patients undergoing assisted reproductive technology (ART) procedures highlighted that reduced MI:DCI ratios result in worse oocyte quality [16,17], and that combined treatment with MI and DCI (40:1 ratio) improves fertility outcomes [18,19]. All these findings confirm that the dominant follicles necessitate of a higher MI content, but also suggest that the organism regulates physiological processes by controlling the MI:DCI ratio.

Effect of D-chiro-inositol on ovarian steroidogenesis

Besides mediating the intracellular message of insulin [20], DCI independently influences the androgen production. Indeed, earlier studies from Nestler and co-workers demonstrated that DCI (in the form

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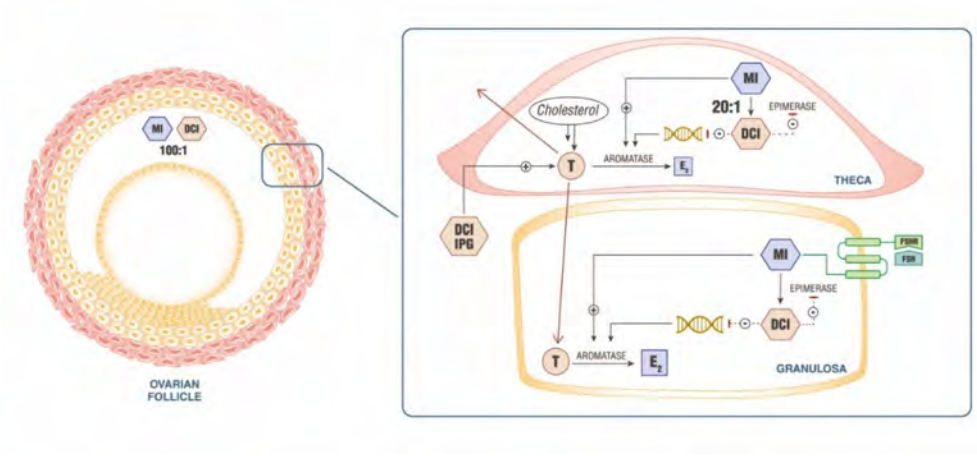

Fig. 1. Schematic depiction of the suggested roles of MI and DCI in the steroidogenesis of thecal and mural granulosa cells. MI: myo-inositol and phosphorylated derivatives; DCI: D-chiro-inositol and phosphorylated derivatives; DCI-IPG: DCI-based phosphoglycan; T: testosterone; E_2: estradiol; FSH: follicle-stimulating hormone; FSHR: FSH receptor. The follicular fluid of healthy dominant follicles exhibits MI:DCI ratios in the range 100:1–70:1. While MI:DCI ratio drops to around 20:1 in the thecal cells, literature data are unavailable for the granulosa. A negligible fraction of testosterone produced in the theca is converted to estradiol. Testosterone is rather transported to the granulosa cells, according to the two-cells, two-gonadotropin theory. MI:DCI ratios likely modulate the activity of aromatase in the two cell types, determining the steroid production.

of phosphatidyl-glycan) increases the biosynthesis of testosterone in the thecal cells [21]. The same author also observed that DCI reduces the activity of aromatase [22], later leading to a comparison between the effects of DCI and those of aromatase inhibitors [23]. Indeed, short-term treatment (6 weeks) with a very high dose (2400 mg/day) of DCI proved to increase testosterone levels in PCOS women [24]. Only recently, Sacchi and co-workers rationalized such observations, proving that DCI downregulates the gene expression of aromatase in a dose-response manner [25]. Available evidence indicates that both MI and DCI regulate steroidogenesis, with opposite effect on aromatase activity: higher MI:DCI ratios favor the activity of aromatase, inducing estrogen biosynthesis; lower MI:DCI ratios favor the production of androgens. Studies on porcine model revealed that follicles that undergo atresia contain significantly higher levels of androgens than the dominant follicle [26]. Moreover, a decreased activity of aromatase can be observed both in the theca and the granulosa of these follicles. While declining FSH levels may account for the effect on the granulosa cells, the reduced aromatase activity in the theca must be FSH-independent as thecal cells lack receptors for FSH.

The hypothesis: MI-to-DCI ratio in selecting the dominant follicle

On these premises, we put forward the hypothesis that the regulation of MI:DCI ratio may define the follicles' fate in the following way. At the beginning of the menstrual cycle, elevated FSH levels stimulate the development of the entire cohort of follicles, independently of their MI content. In response to estrogen production, FSH decreases falling below a certain threshold (around day 6), when only the follicle with the highest content of MI can continue to sustain its stimulus. All other follicles enter the process of atresia. Our hypothesis would also provide a rationale to explain why FSH stimulation in ART protocols yields a higher number of mature oocytes, but only some of them are of viable quality [17].

Testing the hypothesis

This hypothesis implies the presence of a higher MI:DCI ratio in the dominant follicle, with respect to follicles that undergo atresia. We expect to observe such difference mostly in the granulosa cells, as they are the major target of FSH in the selection process. At the present stage, the entity of the differences in the thecal cells and in the follicular fluid is unfathomable.

To validate our hypothesis, inositol content should be assessed both in dominant and atretic follicles. While the literature provides indications about the inositol ratio in the thecal cells and the follicular fluid of dominant follicles, the MI:DCI ratio in the granulosa might be determined in cultured cells retrieved from healthy unstimulated volunteers. Assessing the inositol ratios in atretic follicles will provide the ultimate confirmation of our theory, but it may possibly require the development of an ultrasonography-based method to identify follicles that undergo atresia among the recruited cohort.

Conclusions

The entire cohort of recruited follicles unquestionably contributes to the reproductive process: while the dominant follicle provides the oocyte for fertilization, those that undergo atresia are responsible for increased sexual desire. Based on available evidence in the literature, we speculated that epimerase activity, by defining the MI:DCI ratio in ovarian follicles, may determine their fate. Considering this novel perspective, we would like to spark debate on the mechanism of ovarian folliculogenesis, possibly prompting researchers to undertake studies that address the following questions: *(1)* does epimerase regulation depend on follicle genetics?; *(2)* is the recruitment of follicles driven by their different capability to regulate the epimerase activity? Answering such questions would lead to establish whether the recruited follicles have a pre-determined destiny and to a deeper insight in the mechanisms behind folliculogenesis. Thorough understanding of the ovarian physiology allows to tailor the controlled stimulation procedure during ART protocols, and to identify the appropriate therapeutic approaches for pathological conditions.

Authors' roles

Both authors equally contributed to write and revise the present manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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