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A Review of Biochemical Metabolites Concentration and Hormonal Composition of Ovarian Follicular Fluid in Domestic Animals

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Follicular fluid (FF) is an avascular compartment within the mammalian ovary separated from the perifollicular stroma by the follicular wall that constitutes a blood-follicle barrier. FF plays a major role in the autocrine and paracrine regulation and also in the physiological, biochemical and metabolic aspects of the nuclear and cytoplasmic maturation of the oocyte and the process of ovulation. This fluid is also composed of locally produced substances within the follicle, which are related to the metabolic activity of follicular cells, and it is also in part an exudate of serum. The ovarian FF provides suitable microenvironment for the development, growth and maturation of the oocyte and is vital for the maintenance of fertility in the female. FF protects the oocyte from factors that induce premature resumption of meiosis, guards the oocyte from proteolytic attack, facilitates its extrusion during ovulation, and enhances sperm attraction, motility, and the acrosome reaction.

Keywords: Domestic animals; follicular fluid; metabolites; hormones.

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1. INTRODUCTION

Follicular fluid originates mainly from the peripheral plasma by transudation across the follicle basement lamina and accumulates in the antrum formed by the coalescence of small pockets of fluid [1]. FF could be used as an effective media supplement for *in vitro* maturation as it influences the ability of in vitro-matured oocyte to acquire developmental competence [2]. The advantages of incorporation of FF on *in vitro* oocyte maturation are due to the presence of growth factors, follicle stimulating hormone (FSH), luteinizing hormone (LH) and several nutrients in whereas the disadvantage is it being in the presence of oocyte maturation-inhibitory factor [3,4]. The knowledge of the biochemical composition of FF can also provide useful information about the requirements for cell and oocyte growth and maturation [5,6]. Moreover, such information may be used as a provisional guide for formulating suitable culture media for in vitro cell culture and oocyte maturation in a particular species [7]. Changes in the biochemical composition of FF may influence steroidogenesis, oocyte maturation and quality, ovulation and transport of the oocyte to the oviduct, as well as the preparation of the follicle for subsequent corpus luteum formation and function [8]. Several *in vitro* studies have shown that metabolites in FF may influence the competence of bovine oocytes to mature and, after fertilization, to grow to the blastocyst stage [9,10,11]. Biochemical metabolites concentration in the FF of the bovine ovary fluctuate considerably with the stage of cycle, follicle size and follicle status and presence of large follicles [1,12,13]. Leroy et al. [14] demonstrated that the oocyte and the granulosa cells grow and mature in a changing biochemical environment from small to large follicles. In the previous studies FF concentration of biochemical metabolites such as glucose, cholesterol, triglycerides, total proteins, albumin and globulin have been determined in camel, cattle [15,16] and sheep [50]. These researchers demonstrated that the FF concentrations of biochemical metabolites changes from small to large follicles. There are many studies that suggesting a possible local effect of the corpus luteum on follicular growth and follicular composition [24,51,67].

It has been known that FF is rich in steroid reproductive hormones including testosterone, estradiol and progesterone [11,30]. FF and steroid hormones are commonly used in the culture media as supplements for oocyte maturation; thus, steroid concentrations in the follicular fluid need to be quantified [32,36]. In most mammalian species, estrogen (E_2) dominates the pre-ovulatory follicular environment and meiotic division is resumed shortly before ovulation as a consequence of the pre-ovulatory LH surge [41]. Progesterone (P_4) is the key hormone for maintaining pregnancy, and its production is initiated and remains low in the follicles prior to ovulation [41]. The androgen-estrogen ratio in the follicular fluid reflects the physiologic integrity and variability of the follicle. Information on the concentrations of steroids, especially P_4, E_2 and testosterone which are important for the maturation of follicle [6], in the FF has been carried out by several researchers [6,32,66]. Varying degree of steroid hormonal concentration in the FF is related to size, growth of follicle, stage of estrus cycle and healthy and atretic state of the ovarian follicles [42,52,32,66]. In the previous study Atheya and Totey, [6] demonstrated that the information on the concentrations of steroids hormone is important for the maturation of follicle. On the other hand, Rahman et al. [55] reported that the concentrations of E_2 and P_4 increased with the growth of the follicles. In addition, Yu et al. [74] reported that the steroid hormones in the serum and FF are one of the major factors controlling follicular development. Thyroid hormones are required for bovine ovarian follicular function [5]. Triiodothyronine (T_3) has been shown to synergize with FSH to induce differentiation of granulosa cells in porcine follicles [46,47] and the treatment with thyroxin (T_4) enhanced antral follicular development and ovulation rates in rats [56]. Blaszczyk et al. [14] demonstrated that bovine FF contains the free fractions of thyroid

hormones. It is well known that thyroid hormones play a key role in cholesterol homoeostasis [27], and that the conversion of cholesterol to pregnenolone is the first enzymatic step in the biosynthesis of all steroid hormones [26,14]. Spicer et al. [60] evaluated the effects of thyroid hormones at the level of follicular cells and it was shown that T_3 and T_4 positively contributed to LH induced androstenedione production by bovine theca cells. Additional results also suggested that T_4 alone has a positive impact on FSH-induced progesterone production by bovine granulosa cells [60].

2. FOLLICULAR FLUID

FF is composed partly of secretions from the follicle, and partly of exudates from plasma. Its composition reflects changes in the secretory processes of the granulosa layer and theca interna, and alterations in the components of plasma due to physiological or pathological processes [21]. The presence of FF in so many species testifies to its potential importance in ovarian physiology, including steroidogenesis, growth of the follicle and ovulation, and maturation of the oocyte and its transport to the oviduct. The fluid can be collected easily, especially as the follicle enlarges, and there has been prolonged interest in its composition and functions [20]. FF provides a very important microenvironment for the development of oocytes. It is reasonable to think that some biochemical characteristics of the FF surrounding the oocyte may play a critical role in determining oocyte quality and the subsequent potential to achieve fertilization and embryo development. The analysis of FF components may also provide information on metabolic changes in blood serum, as the circulating biochemical milieu may be reflected in the composition of FF [44].

3. STEROID HORMONES IN FOLLICULAR FLUID

FF has for many years been known to contain steroids [20]. Detailed studies on the nature of the steroids and their turnover rates have been more recent as analytical methods have been improved, and as the relative steroidogenic activity of the theca interna and membrana granulosa has been recognized [17]. Assays of steroids in FFs are currently being used to examine the growth of follicles during natural and induced cycles. Elevated E2 and E2/P4 ratio in FF indicate a more advanced stage of oocyte maturation and have been repeatedly found to be associated with a higher chance of achieving pregnancy [63,64]. There is also conflicting evidence regarding the meaning of P_4 levels in FF. Several authors found that a high FF P4 concentration (or a low E2/P4 ratio) was predictive of subsequent implantation and pregnancy [10,69], and considered it as a reflection of progressive follicle luteinization and reduction of aromatase activity linked to the achievement of the final oocyte maturation. On the other side, however, oocytes from follicles having high FF P4 were frequently found in association with postmature oocytes that fertilized abnormally and gave rise to multipronuclear embryos [12]. It seems that while an optimal exposure to P4 has positive effects over oocyte characteristics, an excessive exposure leads to a rapid worsening of the cell's quality; a clear knowledge of the threshold at which P4 begins to damage the oocyte is presently lacking. Elevated FF androgen levels (testosterone) were associated with lower quality oocytes, and in particular with oocytes showing a trend toward lower cleavage rates after fertilization [68]. E2/T ratio was also reported to be higher in pregnancy-associated follicles [73]. Taken together, these data seem to indicate that a low E_2/androgen ratio in FF may be associated with early follicular atresia, which negatively affects the viability of the enclosed oocyte and limits the chances of fertilization and pregnancy. Indeed, the notion that a predominantly androgenic intrafollicular environment may lead to follicular atresia is well accepted, but at the same time it is widely accepted that a certain amount of intrafollicular

androgens is needed to obtain optimal follicular growth. In fact, in IVF cycles with unsatisfactory ovarian response to FSH, addition of LH (and thus stimulation of androgen synthesis by theca cells) has been found to improve follicle growth and oocyte maturation [18,45].

4. THYROID HORMONES IN FOLLICULAR FLUID

Thyroid hormones govern the control of growth, differentiation and metabolism in nearly all somatic tissues [34]. Due to their general metabolic and permissive effects, thyroid hormones are essential for normal ovarian activity in mammalian species. Abnormal thyroid hormone levels have been reported to lead to infertility or reduced reproductive function [29]. Studies that manipulated systemic thyroid hormone levels to produce a hypothyroid state in dogs have resulted in decreased sensitivity of ovarian theca and granulosa cells to gonadotropins [54]. Spicer et al. [60] evaluated the effects of thyroid hormones at the level of follicular cells and it was shown that T3 and T4 positively contributed to LH-induced androstenedione production by bovine theca cells. Additional results also suggested that T4 alone has a positive impact on FSH-induced progesterone production by bovine granulose cells [60]. Since the 1920 it was known that the iodine concentration in mammalian species was higher in the ovary than in every other organ except for the thyroid gland itself [59]. The presence of thyroid hormone in human FF was demonstrated by Wakim et al. [70] and Zhang et al. [75]. Maruo et al. [47] showed that thyroid hormone synergizes FSH to induce the differentiation of granulosa cells from porcine follicles. Moreover, thyroid hormones directly alter granulose cell steroidogenesis in pigs [46] and humans [75].These findings are further supported by the identification of thyroid hormone receptors and/or their mRNA in porcine granulosa cells [47] and human granulosa cells, cumulus cells and oocytes [75]. Furthermore, the results of Spicer et al. [60] support the hypothesis that thyroid hormones may have direct stimulatory effects on ovarian function in cattle, acting at the level of granulosa and theca cells. However, the exact mechanism by which the thyroid hormones regulate steroidogenesis in bovine follicle is not well known. The data reported by Nixon et al. [53] showed that concentrations of serum T4 in cows were from 1.37 ng/dl in early lactation to 1.85 ng/dl in midlactation. Similar values were reported also in blood plasma of goats following induced oestrus in spring [13]. Zhang et al. [75] demonstrated that the concentrations of T4 in human FF were in the same range as those found in human serum. In addition, the values for T4 concentrations in bovine FF were similar to those reported in human follicles [75]. The differences in levels of T4 between groups of follicles may be related to growth and maturation of follicles in cattle. It is likely that these differences may reflect a greater conversion of triiodothyronine (T3) to thyroxine (T4) in medium and large follicles.

5. BIOCHEMICAL METABOLITES IN FOLLICULAR FLUID

FF is in part exudates of serum and is also partially composed of locally produced substances, which are related to the metabolic activity of the follicular cells [23]. This metabolic activity, together with the barrier properties of the follicular wall, is changing significantly during the growth phase of the follicle [25]. Therefore, a different biochemical composition of the FF in different sized follicles can be expected. Metabolic changes in blood serum may be reflected in the biochemical composition of FF [44]. As the oocyte and granulosa cells grow and get mature in a biochemical environment that changes from small to large follicles, the metabolite, ion, and enzymatic characteristics of FF and follicle or oocyte development are highly correlated [35]. Tabatabaei and Mamoei [61] reported that

the glucose concentration in small follicles was significantly lower than that measured in medium and large follicles. Leese and Lenton [43] has stated that glucose concentrations in FF in women is a result of both glycolysis taking place in mural granulosa cells and influx of same molecules from the plasma into the fluid. Leroy et al. [44] observed that the concentration of glucose in FF of small follicles was less than half of the level found in serum but only 21% lower in large follicles. Leroy et al. [44] also reported that the FF glucose concentration was closely correlated with the serum levels and that it was consistently higher than in serum, possibly due to an active inward transport. This finding strongly suggest that post partum changes in glycemia are well reflected in the FF of dominant follicles but that the oocyte is more or less protected from low glucose concentrations.

Nandi et al. [50] reported that the cholesterol concentration increased with an increase in follicular size, which was in agreement with the findings of Bordoloi et al. [15], Thakur et al. [65] and Mishra et al. [49] in goat and Brantmeier et al.[16] in cattle. Cholesterol in FF derived from two sources, cellular de novo synthesis from acetate and uptake from plasma lipoprotein. Cholesterol in the FF was in the form of a constituent of high-density lipoprotein [16]. Cholesterol was the precursor for steroid synthesis and the FF contained only high-density lipoprotein, therefore, the avascular granulosa cells of the follicles totally depended on the cholesterol from high-density lipoprotein, which was derived from the blood plasma by crossing the basement membrane of granulosa cells [49]. As the production of steroids increased, the follicle's level of cholesterol also increased [72]. The result of Nandi et al. [50] differed with reported in pigs [33] and in buffalo [66]. The decreased cholesterol level in the large follicles in those studies might be attributed to the conversion of cholesterol to steroid hormones, estrogen and progesterone during steroidogenesis. Leroy et al. [44] reported that the total cholesterol in FF was about 42% of the concentration found in blood serum and there was a significant increase of the total cholesterol content from small to large follicles. Cholesterol, present in FF, is bound to the high-density lipoprotein fraction (HDL) because the only other cholesterol-containing lipoprotein fraction, the low-density lipoprotein fraction (LDL), is too large to pass the blood–follicle barrier [27]. The higher total cholesterol concentration in large follicles can be explained by the increased permeability of the follicular wall in that follicle class, permitting the entrance of the larger HDL fraction [8,71]. The triglycerides concentration was higher in small follicles because might be the alternate sources of energy for the cells in follicles [44]. Another reason for the high concentrations of triglycerides in small follicles was that triglycerides did not pass through the follicular membrane [27] and follicular triglyceride levels were mainly a result of local metabolic processes [44]. Leroy et al. [44] also reported that the amount of triglycerides decreased from small to large follicles. Triglyceride levels in small follicles were significantly higher than in serum and significantly lower in large follicles. A relatively stable concentration of triglycerides is maintained in the bovine ovarian follicle, regardless of increases in serum due to physiological status or diet [71]. Triglycerides probably do not pass through the follicular membrane since they are transported primarily by the very low-density lipoprotein fraction (VLDL), which is too large to pass through this barrier [27]. In FF, triglycerides may serve as an alternative energy source since cells cultured in vitro can absorb and consume triglycerides out of the medium. Also, oocytes and embryos show lipid accumulation when cultured in triglyceride containing media [40,2]. The total protein content of the FF was determined in the studies in goats [58], cattle [44] and in sheep [9]. Unlike the observations of Arshad et al. [4] and Leroy et al. [44], total protein concentration in fluid of small follicles was significantly higher than that in large follicles. In the study of Tabatabaei and Mamoei [61] the concentration of protein in blood plasma was significantly higher than the levels in all follicle classes, which was similar to results of Arshad et al. [4] and Leroy et al. [44]. In swine, total proteins concentration in the fluid from small follicles was slightly higher than in

fluid from large follicles, while the serum had non-significantly higher total proteins concentration compared with small and large follicles [48]. Rahman et al. (55) reported that the serum contained less albumin and a globulins compared with the FF, while gamma-globulins were higher in serum than in the FF.

6. CONCLUSION

From the present review article it was concluded that the hormonal and biochemical metabolites concentration in the FF fluctuate considerably with the stage of cycle, follicle size and follicle status and presence of large follicles. In addition, the ovarian FF provides suitable microenvironment for the development, growth and maturation of the oocyte and is vital for the maintenance of fertility in the female animal.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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