JBRA Assisted Reproduction 2016;**20**(1):38-40
doi: 10.5935/1518-0557.20160009

Review Article

Human follicular fluid and effects on reproduction

Laís Basuino[1], Carolina F. Silveira[1]

[1]Sapientiae Institute - Study and Research Center, São Paulo, SP, Brazil.

ABSTRACT
Fertility – the ability to produce offspring – is considered a prerequisite for the development and perpetuation of species. Several factors may positively or negatively affect one's reproductive capabilities, such as regular exercises and maintaining a healthy bodyweight, versus aging, obesity, and stress.
Follicular fluid (FF) is a liquid composed primarily of hormones, enzymes, anticoagulants, electrolytes, reactive oxygen species and antioxidants, which fills the follicular antrum and acts as an important mediator in the communication between cells in the antral follicle while bathing and carrying nutrients to the oocyte. Thus, human FF is a key element to the success of natural fertilization present in every stage of the conception process, from the communication between gametes to the development of fully viable embryos, and a vital component in the occurrence of spontaneous pregnancies. This literature review aimed to describe the possible effects of human follicular fluid on the natural fertilization process and to assess its role in follicular growth, oocyte quality, sperm capacitation, fertilization, and early embryonic development.

Keywords: Human follicular fluid, antral fluid, fertilization, sperm capacitation.

INTRODUCTION
Fertility – the ability to produce offspring – is considered a prerequisite for the development and perpetuation of species (Simões, 2010).

Several factors may positively or negatively impact one's childbearing capacity to greater or lesser extents. Age may be a factor for women aged 30 or older, and to a lesser degree to men above the age of 35 years. Additionally, poor nutrition, obesity, stress, caffeine intake, drinking, smoking, using illicit or medicinal drugs, exposure to radiation, heavy metals, toxic chemicals and pollution may negatively affect patients (Sharma *et al*., 2013).

On the other hand, regular exercises, maintaining a healthy bodyweight, and having good eating habits throughout life may protect one's fertility and prevent decreases in reproductive function quality (Sharma *et al*., 2013).

Fertilization occurs when the male and female gametes meet in a complex process that involves several steps and the participation of many factors. The process, however, is quite similar among mammals and is accurately regulated by species-specific gamete recognition mechanisms. In humans, the process unfolds through various biochemical reactions and with the aid of glycoproteins present in the male ejaculate responsible for the interaction between gametic cells (Kratz & Achcinska, 2011; Sullivan & Saez, 2013).

The antral follicle shelters the oocyte. For the oocyte to be fit for fertilization it must reach the correct maturation stage and have completed the processes of growth, capacity and nuclear and cytoplasmic maturation, in a complex and dynamic relationship developed between oocyte and the antral follicle. The follicle features a specialized microenvironment, responsible for ensuring the production of female gametes with quality and integrity, providing support and supplying needs of the oocyte until it is released for fertilization in the uterine tube (Hennet & Combelles, 2012).

The antral follicle comprises a specialized layer of extracellular matrix that surrounds the entire follicle called the basal lamina, and two different types of somatic cells forming the layers of the internal and external theca cells. These layers are vascularized and delimit the follicle size. The oocyte is protected by granulosa cells in the inner follicular lining, forming the cumulus-oocyte complex (COC). These cell layers play an active role in the regulation of oocyte maturation. Gaps in the junctions of the COC allow the passage of ions, metabolites, amino acids, glucose and signaling molecules from the cumulus cells to the oocyte, providing nutrients and energy substrates needed in the development of the oocyte. At the center of the follicle there is a cavity known as the follicular antrum, which is completely filled by a liquid known as the follicular fluid (FF) (Hennet & Combelles, 2012).

Ovarian folliculogenesis requires a constant balance between the regulating extra and intra-ovarian factors to ensure that the process occurs correctly. Oogenesis also depends largely on the performance of these factors – particularly the ones present in the FF. Changes in the relationship between these factors might result in abnormal folliculogenesis and poorer-quality oocytes (Resende et al., 2010).

The FF is formed from two sources, namely the bloodstream, connected to some thecal capillaries present in the cortical region of the ovary, and the components secreted by the cell layers that are within the follicle, especially the granulosa cells (Rodgers, 2010; Hennet & Combelles, 2012). At the moment of natural ovulation, a large amount of FF is expelled with the oocyte toward the uterine tube (Wilding *et al*., 2006).

This fluid is primarily composed of protein and steroids hormones, including follicle stimulating hormone (FSH), luteinizing hormone (LH), growth hormone (GH), human chorionic gonadotropin (hCG), progesterone and estradiol; cytokines; enzymes; anticoagulants; electrolytes; reactive oxygen species (ROS); enzymatic and non-enzymatic antioxidants, including vitamin E, catalase and melatonin; growth factors such as epidermal growth factor (EGF), EGF like growth factor (EGF-like) and transforming growth factor alpha (TGF-α); metabolites such as amino acids and lipids that accumulate within the oocyte, helping its differentiation; and fatty acids (Hsieh, Zamah, Conti, 2009; Resende *et al*., 2010; Hennet & Combelles, 60. 2012; Shaaker *et al*., 2012).

These compounds are important mediators of the communication between different types of cells in the antral follicle, in addition to transporting elements and nutrients to the oocyte and bathing the oocyte and the fimbria of the uterine tube near the ovary (Hennet & Combelles, 2012; Emori & Drapkin, 2014). To do so, the FF must have good fluidity so that the supply of nutrients to the oocyte is constant and sufficient to ensure its good quality. Therefore, the FF must have a good interaction with the transport molecules present in the cells surrounding each of

Received October 01, 2015
Accepted January 27, 2016

the oocytes (Rodgers, 2010). These qualities also allow the use of FF as a supplement to in vitro fertilization medium (Shaaker *et al.*, 2012).

MATERIALS AND METHODS

This literature review included relevant scientific publications on the subject matter available in databases PubMed and SCielo. The search was performed using keywords in Portuguese and English. The selected papers were published within the last ten years and included some historical content on the subject. Literature reviews and experimental works were included.

Words used in the search: Human follicular fluid, antral fluid, fertilization, sperm capacitation.

RESULTS

The initial search yielded a list with 25 papers, of which 12 were selected. Eight were literature reviews, three were experimental studies, and one was a case-control study. The selected studies discussed the importance of human FF, its elements, and impact in steps contributing to successful fertilization.

According to Table 1, FF has a more prominent role in the maturation and oocyte quality steps, indicating a constant communication between the fluid and the oocyte during its development. The two events were cited in seven of the 12 selected papers. Follicular growth and fertilization were cited in six papers each; the oocyte and sperm capacitation steps were mentioned in five papers each; early embryonic development was mentioned in four papers; and oocyte nutrition in one of the papers.

The selected literature reviews generally addressed the formation and activity of the FF, its importance, and connection with the fertilization process, while eliciting the main FF components and the ways in which each operates and influences the various necessary steps within the processes required for fertilization and development of a healthy embryo.

In the experimental study by Getpook & Wirotkarun (2007), sperm motility preservation and stimulation was assessed at FF concentrations ranging from 20% to 100%. The authors found that all evaluated semen samples had significant sperm motility stimulation in all tested FF concentrations, and that the effect remained for up to 12 hours after the start of stimulation analysis. Control group samples not given FF had unsatisfactory motility results.

Caille *et al.* (2012) looked into the mechanisms involved in the acrosome reaction induced by FF and the possible influence of progesterone in this process in sperm that had been previously treated with peritoneal fluid. The authors found that the progesterone present in FF was responsible for inducing this reaction. The authors also concluded that even in the presence of FF and progesterone, the peritoneal fluid, which also plays a role in the sperm capacitation process, may exert an inhibitory effect on sperm, thus reducing the capacity of these gametes to respond to stimulus for the acrosome reaction.

Shaker and collaborators (2012) assessed FF samples taken from a group of 100 women undergoing assisted reproduction cycles. The authors used chromatography to analyze saturated, mono and polyunsaturated fatty acids present in these samples. They observed that these fatty acids in FF interacted directly with the oocyte, with beneficial or detrimental effects depending on whether their levels were appropriate or very high in each cycle.

The case-control study published by Resende *et al*. (2010) looked into the testosterone, androstenedione, estradiol, progesterone and β-hCG levels present in the FF aspirated from the small and large follicles of two groups of women undergoing assisted reproduction cycles. The case group comprised individuals diagnosed with Polycystic Ovary Syndrome (PCOS) and anovulatory cycles, while the control group had women with ovulatory cycles and mild male factor infertility. The differences found in the levels of each element were due to the difference in the sizes of each follicle during the cycle and occurred in both groups of patients. The levels of testosterone and progesterone were altered in the FF of women with PCOS when compared to women with ovulatory cycles, whereas the other three elements analyzed had similar levels in the individuals of both groups.

DISCUSSION

The data show that the FF contributes positively to the set of studied events, particularly to the development of oocytes with good quality and the formation of a properly capacitated sperm subpopulation to enable successful subsequent natural fertilization.

The role of the FF in oocyte and sperm development for purposes of fertilization was presented and discussed in the selected papers.

The analysis of metabolites produced by the FF may be used as a strong noninvasive marker of oocyte quality, in addition to shedding light on the interaction between the FF, the oocyte, and the elements that determine and aid in proper oocyte development. The FF was shown to have direct impact on the growth and maturation of oocytes – factors deemed essential for good fertilization and the development of good embryos (Revelli *et al*., 2009; Rodgers & Irving-Rodgers, 2010; Ola & Sun, 2012; Hennet & Combelles, 2012).

Glycodelin is present in the FF and can also be found in the granulosa cell layer and in the uterine tubes. Glycodelin works as an immunosuppressant and protects the sperm against possible responses from the female immune system. The gamete fertilization potential is increased when there is a good interaction between sperm and glycodelin. Furthermore, this protein prevents an early acrosome reaction, assists in nutrition and sperm capacitation, influences the selection of sperm with better morphology and the migration of sperm towards the oocyte for subsequent fertilization (Seppälä *et al*., 2007).

The FF is responsible for stimulating the acrosome reaction and preserving and improving sperm motility, in addition to playing a role in the sperm adaptation process in the final stage preceding fertilization (Getpook & Wirotkarun, 2007). The progesterone present in the FF also assists in the occurrence of the acrosome reaction as well as in sperm hyperactivation and chemotaxis (Caille *et al*., 2012).

The peritoneal fluid filling the peritoneal cavity associated with FF is an active element in the formation of the environment that houses the ovulation process, transport and survival of gametes, oocyte-sperm interaction, early embryonic development and implantation. Furthermore, in sperm capacitation, which occurs as sperm passes by the uterine tube, sperm are first exposed to the peritoneal fluid to then come into contact with the FF. In the acrosome reaction that occurs in vivo, peritoneal fluid also acts as a possible regulator, preventing sperm from suffering an early loss of its acrosome, which would preclude the conclusion of the fertilization process (Caille *et al*., 2012).

Chemotaxis and its relationship with the FF are also important in the conception process. This event allows sperm to be drawn closer to the oocyte to fertilize the female gamete. In this process, the initially circular motion of the tail of the sperm cells must be hyperactivated. During chemotaxis, the sperm cell becomes faster, stronger and with wavy movements in the tail (Yoshida & Yoshida, 2011).

In the case of humans, the attraction regulator is not

Table 1: Studies addressing the role of the follicular fluid (FF) in events related to fertilization.

Authors	F.G.	Ovulation	O.N.	M.Q.	S.C.	Fertilization	E.E.D.
Seppälä *et al.* (2007)					x		
Getpook & Wirotkarun (2007)**					x	x	
Revelli *et al.* (2009)	x	x		x		x	
Rodgers & Irving-Rodgers (2010)	x	x					
Resende *et al.* (2010)*	x	x		x			x
Fujimoto *et al.* (2010)				x		x	x
Yoshida & Yoshida (2011)					x	x	
Caille *et al.* (2012)**					x		
Shaaker *et al*. (2012)**				x	x	x	x
Hennet & Combelles (2012)	x	x	x	x			
Ola & Sun (2012)	x			x			
Tamura *et al*. (2013)	x	x		x		x	x

Legend: X – FF contributes to the occurrence of the event; Empty – the author has not established any correlations between the FF and the event; F.G. – Follicular growth; O.N. – Oocyte nutrition; M.Q. – Maturation and oocyte quality; S.C. – Sperm capacitation; E.E.D. – Early embryonic development; (*) – Case-control study; (**) – Experimental study.

the oocyte, but the follicular fluid with the participation of progesterone and calcium present in the intracellular medium. About 2% to 12% of the ejaculated sperm have a chemotactic response to progesterone and this is the sperm subpopulation considered able for fertilization (Yoshida & Yoshida, 2011).

Metabolic action of the FF also includes the participation of High Density Lipoprotein (HDL), one of its most abundant components. The presence and amount of these particles in the FF are influenced by the follicular basal lamina and by perifollicular vascularity. This hematofollicular barrier acts as a filter, allowing the selective diffusion of various proteins and small molecules (Fujimoto *et al*., 2010).

This lipoprotein contributes to the intra-follicular homeostasis of cholesterol, serving as a substrate for the synthesis of progesterone. HDL also assists actively in good oocyte and embryo development, being positively related to the cleavage rates of embryos in their early stages of growth and not associated with embryonic fragmentation (Fujimoto *et al*., 2010).

While the FF works as a microenvironment of the antral follicle, the presence of antioxidants and reactive oxygen species in this medium allows these two elements to participate in the process of interrupting and resuming the phases of meiosis, thereby modulating the maturation of oocytes (Hennet & Combelles, 2012). The HDL particles in the FF can moderate the action of free radicals, thereby limiting oxidative damage in cells and other lipoproteins (Fujimoto *et al*., 2010).

However, in women who have difficulty getting pregnant and diagnosed with endometriosis, for example, the antioxidant activity of the FF occurs less efficiently than in women with PCOS or diagnosed with tubal occlusion alone. When the ability of FF to inhibit lipid peroxidation is impaired, there may be a decline in oocyte quality in patients with endometriosis. Increases in the levels of oxidative stress in the FF may also impair fertilization capacity and embryo development (Huang *et al*., 2014).

After being secreted by the pineal gland, the melatonin that comes into contact with the FF through the bloodstream works in reproduction as an antioxidant with higher concentrations in larger follicles. Melatonin can reduce cellular oxidative stress and contribute to oocyte maturation, while participating in the process of ovulation, luteinization of granulosa cell layer, early embryonic development and progesterone production by the corpus luteum, in addition to increasing fertilization and pregnancy rates (Tamura *et al*., 2013).

The fatty acids contained in the phospholipids of the FF may be absent at certain times as their levels vary. These changes reflect the eating habits of women during the different seasons of the year, and therefore impacting metabolism and fertility. The levels of saturated fatty acids found in the FF are higher in oocytes and granulosa cells in the summer season while the levels of mono and polyunsaturated fatty acids are higher during winter, as a consequence of changes in eating habits throughout the year (Shaaker *et al*., 2012).

These fatty acids come into contact with the oocyte through the FF circulation and are incorporated by oocyte phospholipids. Then, they start to contribute to oocyte maturation and quality and early embryonic development, thus improving the success rates of in vitro fertilization procedures. However, in cases of obese or overweight patients who need to lose weight before starting IVF, excessively high fatty acid levels even after the achievement of a healthy body mass index (BMI) may have a detrimental effect on patient fertility. Linoleic acid, for example, has been positively related to oocyte maturation, whereas arachidonic acid has been negatively related to fertilization rates. Despite these variations, fatty acids are very important in human FF metabolism and the phospholipids in it are among the main components of body lipid metabolism (Shaaker *et al*., 2012).

In patients with PCOS, inadequate levels of elements present in the FF such as testosterone, androstenedione, estradiol, progesterone and β-hCG, are indicative of inefficient follicular development and may adversely affect oocyte quality and fertilization rates. One example is when follicles are found to be small and immature (below 14 millimeters in diameter) in a medium with high levels of testosterone. Conversely, higher levels of progesterone in the fluid of large mature follicles (above 14 millimeters in diameter) is due to follicular maturation stage. The low levels of progesterone see in women with PCOS, however, indicate they may have impaired production and secretion of the hormone resulting from damaged follicular cells (Resende *et al*., 2010).

When compared to healthy women, individuals with PCOS have similar levels of androstenedione and estradiol in the FF – regardless of follicle size – and similar pregnancy rates based on β-hCG levels. These findings showed that assisted reproduction techniques might be a good option for women with PCOS having trouble becoming pregnant (Resende *et al*., 2010).

The papers included in this review showed that human FF is a key element in the success of natural fertilization, as it acts in all stages of the conception process, from the communication between gametes to the development of fully viable embryos, thus enabling the occurrence of spontaneous pregnancy. The FF influences mainly oocyte growth, nutrition and maturation, all of which are key factors in fertilization. FF proteomic and metabolomic analyses are important non-invasive tools used to evaluate potential biomarkers that can be used as oocyte quality indicators and predictors for embryo quality and early development. Furthermore, the FF has been directly linked to sperm capacitation as the entity responsible for the regulation and maintenance of male gametes during the main steps of fertilization.

CONFLICT OF INTERESTS

No conflict of interest have been declared.

Corresponding author:
Laís Basuino
Sapientiae Institute - Study and Research Center
São Paulo/SP - Brazil
E-mail: laisbasuino@gmail.com

REFERENCES

Caille AM, Berta CL, Cuasnicú PS, Munuce MJ. Peritoneal fluid modifies the response of human spermatozoa to follicular fluid. Reprod Biomed Online. 2012, 24: 466-73.

Emori MM, Drapkin R. The hormonal composition of follicular fluid and its implications for ovarian cancer pathogenesis. Reprod Biol Endocrinol. 2014;12:60.

Fujimoto VY, Kane JP, Ishida BY, Bloom MS, Browne RW. High-density lipoprotein metabolism and the human embryo. Hum Reprod Update. 2010;16:20-38.

Getpook C, Wirotkarun S. Sperm motility stimulation and preservation with various concentrations of follicular fluid. J Assist Reprod Genet. 2007: 425-8.

Hennet ML, Combelles CMH. The antral follicle: a microenvironment for oocyte differentiation. Int J Dev Biol. 2012, 56: 819-31.

Hsieh M, Zamah AM, Conti M. Epidermal Growth Factor-Like Growth Factors in the Follicular Fluid: Role in Oocyte Development and Maturation. Semin Reprod Med. 2009, 27:52-61.

Huang B, Li Z, Ai J, Zhu L, Li Y, Jin L, Zhang H. Antioxidant capacity of follicular fluid from patients undergoing in vitro fertilization. Int J Clin Exp Pathol. 2014, 7: 2273-82.

Kratz EM, Achcinska MK. [Molecular mechanisms of fertilization: the role of male factor]. Postepy Hig Med Dosw (Online). 2011;65:784-95.

Ola SI, Sun QY. Factors influencing the biochemical markers for predicting mammalian oocyte quality. J Reprod Dev. 2012; 4: 385-92.

Resende LOT, Reis RM, Ferriani RA, Vireque AA, Santana LF, de Sá Rosa e Silva AC, Martins Wde P. [Concentration of steroid hormones in the follicular fluid of mature and immature ovarian follicles of patients with polycystic ovary syndrome submitted to in vitro fertilization]. Rev Bras Ginecol Obstet. 2010; 32: 447-53.

Revelli A, Piane LD, Casano S, Molinari E, Massobrio M, Rinaudo P. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. Reprod Biol Endocrinol. 2009;7:40.

Rodgers RJ, Irving-Rodgers HF. Formation of the ovarian follicular antrum and follicular fluid. Biol Reprod. 2010;82:1021-9.

Shaaker M, Rahimipour A, Nouri M, Khanaki K, Darabi M, Farzadi L, Shahnazi V, Mehdizadeh A. Fatty acid composition of human follicular fluid phospholipids and fertilization rate in assisted reproductive techniques. Iran Biomed J. 2012, 3: 162-8.

Sharma R, Biedenharn KR, Fedor JM, Agarwal A. Lifestyle factors and reproductive health: taking control of your fertility. Reprod Biol Endocrinol. 2013;11:66.

Seppälä M, Koistinen H, Koistinen R, Chiu PCN, Yeung WSB. Glycosylation related actions of glycodelin: gamete, cumulus cell, immune cell and clinical associations. Hum Reprod Update. 2007;13:275-87.

Simões MIT. [Infertility: prevalence]. Completion of Course Work (Undergraduate Medical Education) – Porto University, Porto, 2010.

Sullivan R, Saez F. Epididymosomes, prostasomes, and liposomes: their roles in mammalian male reproductive physiology. Reproduction. 2013;146:R21-35.

Tamura H, Takasaki A, Taketani T, Tanabe M, Kizuka F, Lee L, Tamura I, Maekawa R, Asada H, Yamagata Y, Sugino N. Melatonin as a free radical scavenger in the ovarian follicle. Endocr J. 2013;60:1-13.

Yoshida M, Yoshida K. Sperm chemotaxis and regulation of flagellar movement by Ca2+. Mol Hum Reprod. 2011;17:457-65.

Wilding M, Singer M, Fehr P, Haeberlin F, Roth F, Lachat R, Di Matteo L, Capobianco C, Dale B. The effect of extended culture of cumulus–oocyte complexes in follicular fluid during in vitro fertilisation cycles. J Assist Reprod Genet. 2006;23: 129-36.