Novelty Journals

ISSN 2394-7330

International Journal of Novel Research in Healthcare and Nursing
Vol. 2, Issue 2, pp: (43-48), Month: May - August 2015, Available at: www.noveltyjournals.com

Commentary On: Our Published Reported Case "Human Sperm Can Fertilize Ova Inside the Graafian Follicle before Ovulation" And Review Of Literatures

Zakaria M. Nada

MBB ch, M.Sc., M.D., Chief of OB/GYN department, Benha Teaching Hospital, Benha, Egypt

Abstract: **Inside the human body, many physiological events and basic biological properties are still not yet clearly understood. Moreover, most data are from either *in-vitro* or animal studies, and neither is conclusive as they do not represent the physiological condition in humans.**

This article reviews mainly the information concerning the chemotaxis and ovulation processes and *in-vivo* sites of fertilization including our views after our publication of an *in-vivo* evidence of sperm capability to find and recognize the eggs and also that the mature follicular environment is conducive to fertilization.

Pre-contact sperm-egg communication is probably has a crucial role for mutual recognition. The sperm may penetrate the Graafian follicle or go inside through apertures or canaliculi developed in the follicular wall during the process prior to ovulation.

This field needs further investigations which are now possible in humans through IVF techniques.

Keywords: **Fertilization sites (*in-vivo*); gametes recognition; ovarian pregnancy; ovulation process; sperm chemotaxis.**

1. INTRODUCTION

Inside the human body, many physiological events and basic biological properties are still out of our knowledge scope. Moreover, most data are from either *in-vitro* or animal studies, and neither is conclusive as they do not represent the physiological condition in humans.

The processes of mutual gametes recognition and ovulation process are not yet clearly understood, and moreover, most of the information is from *in-vitro* studies. Also, the pathogenesis of ovarian pregnancy, particularly the intrafollicular or the primary type, is still represent a challenge. It has been proposed that fertilization of ovum on the ovarian surface after follicular rupture **(Hallatt, 1982 and Noujua-Huttunen et al., 1995)** is a possible mechanism of primary ovarian pregnancy. On the other hand, fertilization of ovum inside the ovary, specifically intrafollicular, to the best of our knowledge, was just speculation and it was rejected and disapproved by experimental studies **(Noujua-Huttunen et al., 1995)**. In 2005, we could document in our article **(Nada et al., 2005)** that sperm can fertilize ova inside their Graafian follicles before ovulation.

Accordingly, fertilization of egg either on the ovarian surface (**Hallatt, 1982** and **Noujua-Huttunen et al., 1995)**, or inside the ovary **(Nada et al., 2005)**, may be the suggested mechanisms for the primary ovarian pregnancy. The sperm has to be programmed to find and recognize the egg and to reach there with intact fertilizing capacity.

2. OBJECTIVES

There are two factors behind reviewing the published articles of gametes recognition, fertilization inside the ovary and other related papers. The first factor is the uncertainty and mystery of the pathogenesis of ovarian pregnancy, and the second encouraging factor which may reappraise many data is the recently documented fertilization inside the Graafian follicle before ovulation **(Nada et al., 2005)**.

This narrative review addresses mainly two questions; *what* may be the guiding factors for the sperm to find the egg? And *how* do the sperm go inside the Graafian follicle in this process? There may be no clear answer, but this article may open the discussion and speculation and may encourage more researches that might, at the end, help to solve secretes of sperm behavior, gametes recognition and fertilization process.

What may be the guiding factors for the sperm to reach and recognize the egg?

The published articles regarding recognition of egg by sperm or chemotaxis are conflicting, debatable and are depending on *in-vitro* studies. Aspiration of both mature egg and sperm attached to its zona pellucida from inside the same Graafian follicle **(Nada et al., 2005)** is in-*vivo* human evidence that the sperm can run after and recognize the mature egg.

So, to answer the first question, we may suggest that the sperm can reach and meet the mature eggs through one or more of the following mechanisms: pre-contact communication, collision, instinct, or may be through other unknown mechanisms.

Background: the communication inside the ovarian follicle is well documented, generated by gap junction channels and is terminated near the time of ovulation **(Granot and Dekel, 1998)**. This communication serves as a passage for nutrition and regulatory signals for oocytes before ovulation **(Gilula et al., 1978 and Granot and Dekel, 1998)**.

In most studies, chemotaxis is assumed to be operating through chemoattractant substance(s), either released from the egg **(Ralt et al., 1991; Villanueva-Diaz et al., 1992 and Sun et al., 2005)**, or from the follicular fluid **(Jaiswal et al., 1999; Jeon et al., 2001 and Tacconis et al., 2001)**. However, the molecular basis for this behavior, signaling pathway and the functional consequences are still unresolved. The *in-vitro* studies have suggested that sperm chemoattractants are secreted either prior to ovulation within the follicle, as earlier studies have demonstrated **(Jaiswal et al., 1999; Jeon et al., 2001 and Tacconis et al., 2001)**, or after oocyte maturation outside the follicle **(Sun et al., 2005)**). However, it is observed that not all of the follicular fluids cause sperm accumulation **(Ralt et al., 1991 and Villanueva-Diaz et al., 1992)**.

On the other hand, other studies have demonstrated the absence of chemotaxis between human sperm and ova **(Makler et al., 1995)** or follicular fluid **(Makler et al., 1992)**, and no sperm chemoattractants have been identified **(Eisenbach, 1999 and Kunz et al., 1997)**. Not only that, but the debate and conflict have risen up to the extent that other authors **(Eisenbach and Tur-Kaspa, 1999)** questioned themselves whether the nature may allow fertilization to occur only as a consequence of a chance collision between sperm and egg. However, if the recognition is neither through pre-contact communication nor collision, we may also inquire the role of instinct in mutual gametes recognition, or that inborn instinct may be included already as an adjuvant or as a part of the other proposed mechanisms of gametes recognition.

Comment: in the published article **(Nada et al., 2005)**, traveling of sperm from the vagina to meet and interact with eggs in the supposedly non injured and normally located ovaries and recognizing only the hidden mature eggs in situ in their Graafian follicles before ovulation is highly improbable event to have occurred only as a consequence of a chance collision between sperm and eggs. In addition, accomplishment of the sperm's mission would be difficult or impossible unless there is communication and attractiveness between the two gametes and each gamete is prepared/equipped to fit and suite for its work. So, the success of this task denotes the efficiency of both signal systems from the ovary, specifically from Graafian follicle or egg, and the competence of the receptor mechanisms of sperm. Supporting our view, **Tacconis et al. (2001)** have found that the dyspermic semen samples have an impaired capacity to achieve both capacitation and chemotactic responsiveness to chemotactic signals.

Consequently, we can ratiocinate that pre-contact sperm-egg communication is crucial for mutual gametes recognition and fertilization. As a result, we may add, like other authors **(Eisenbach and Tur-Kaspa, 1999)**, that failure or faulty pre-contact sperm-egg communication may be a causative factor for infertility, and also that interfering with human sperm chemotaxis may represent a new approach to contraception.

In most literatures, chemical communication is the assumed pre-contact signal and it operates through chemoattractant substances **(Ralt et al., 1991; Villanueva-Diaz et al., 1992; Jeon et al., 2001; Tacconis et al., 2001 and Sun et al., 2005)**. Whereupon, as in other literature **(Sun et al., 2005)**, these substances are most probably produced by the mature/maturing oocytes or their surrounding cumulus cells and then diffused/secreted into the follicular fluid of their follicles. Follicular fluid thus becomes a conveyer/conductor of these chemoattractant factors and their concentration gradients may guide/lead the sperm further to the mature oocyte-cumulus complex, or the sperm may trace down the originating source of signal whatever may be the nature of it.

So, finding sperm inside the Graafian follicle attached only to the mature eggs is thus a reasonable event *only* if there is pre-contact communication between the two gametes which are at appropriate stages of their respective sequential development simultaneously. Although the communication mode and its mechanism is not yet clear, critical short time window of observation at appropriately coincidental stages for each gamete may be a cause of conflicting observations/reports in literatures.

Sperm attachment only to the mature eggs: In our reported patient **(Nada et al., 2005)**, there was no sperm attached to the immature eggs that have been aspirated during ovum pick up (OPU). This can be explained by the following possibilities. *First*, sperm may not be able to recognize the immature egg, due to the absence of pre-contact communication between the immature egg and sperm. This may be attributed to absence of functioning signal system in the immature oocyte, or the zona pellucida and the preovulatory channels may not be conductive to the signals due to the compact and narrow intercellular spaces of cumulus-corona, which progressively loosen up and enlarge in size around mature oocytes **(Familiari et al., 1998)**. *Second*, the immature egg may be capable of communicating with the sperm as the mature one, but the sperm may be unable to penetrate the immature cumulus-corona or the zona pellucida may be incapable of interacting with sperm. So, we may suggest, like other authors **(Familiari et al., 1998)**, that cumulus-corona and/or zona pellucida could have facilitating activities related to fertilization. However, in our case **(Nada et al., 2005)**, entry of sperm inside the immature follicle and trials to fertilize the immature oocyte cannot be excluded.

How do the sperm go inside the Graafian follicles?

There is a scarcity of information regarding human ovulation and fertilization processes and furthermore, most of the informations are from animal studies. Our reported case **(Nada et al., 2005)** is in-vivo evidence that the sperm can go inside the Graafian follicle. But, how can the sperm do that? At present, it is difficult to answer this question, but my opinion may add help to come nearer to the secret.

Background: it has been observed in humans that there is a rapid increase in the follicular fluid volume just prior to ovulation **(Leon Speroff et al., 1994)** and over a period of 7 hours prior to its rupture there is no demonstrable changes in the size of the follicle **(de Crespigny et al., 1981)**. The increase in size is unaccompanied by any significant change in the intrafollicular pressure and this is explained by the change in the elastic properties of the follicular wall **(Leon Speroff et al., 1994)**. Also, prior to follicular collapse, it has been observed that there is an initial rapid loss of fluid followed by a flower like-release of the remaining contents **(de Crespigny et al., 1981)**.

In addition, it has been found that human chorionic gonadotropin (HCG) has no immediate effect on intraovarian pressure but, 6 to 8 hours after the stimulus is applied, ovarian contractile activity increases significantly. This enhanced activity persists for several hours before returning to initial levels approximately 15 to 18 hours after the HCG injection **(Virutamasen et al., 1976)**. In other studies, it has been documented through electron microscope that there is clearly increase in size of lysosomal bodies up to 8 hours after HCG injection, then decrease markedly. A maximum accumulation of lysosomes is found in the apical epithelium of the Graafian follicle. These lysosomal bodies are transformed into vacuoles which communicate with each other and with the extracellular space *below* the surface epithelium of the follicular wall **(Cajander and Bjersing, 1976)**.

Furthermore, it has been observed that at 13 hours after HCG injection, the beginning of rupture sites formation can be seen in the apices of unovulated follicles. Surface epithelial cells start to be stretched apart with the appearance of gaps between cells, and more often followed by missing portions of surface epithelium and exposure of the underlying cell layers. Taken together these observations demonstrate that intrafollicular pressure in preovulatory follicles decreases gradually at the time follicular smooth muscle cells contract. This is illustrated by the slow and gradual process of

contractions which cause formation and/or enlargement of holes in the rupture site of the Graafian follicle enough in number and size to accelerate the decrease in the intrafollicular pressure. Fluid and erythrocytes can pass through these developed holes or apertures **(Schroeder and Talbot, 1982)**. Also, during that period there is an augmenting edema occupying whole ovary **(Bjersing and Cajander, 1975 and de Crespigny et al., 1981)** and other features that are characteristic of tissue responses to inflammatory reactions **(Espey, 1994)**.

Comment: the sperm to overcome all the obstacles and reach inside the follicles with intact fertilizing status is hard to comprehend. How did the sperm get over the follicular wall barrier and penetrate the layers of granulosa cells, cumulus cells and zona pellucida? This may be extraordinary/exceptional behavior, or can be ordinary/normal event!

The logical thinking, supported by the literatures, is that the sperm may pass through apertures or pores in the follicular wall which are enough in size to allow the sperm to go inside and reach the cumulus-oocyte complex.

To approach this premise, we have to remember that the ovulation process is not an explosive event, and a complex series of changes must occur which cause the final maturation of oocyte and the decomposition of the collagenous layer of the follicular wall **(Bjersing and Cajander, 1975; Morioka et al., 1989; Leon Speroff et al., 1994 and Schroeder and Talbot, 1998)**.

The picture of Graafian follicles after 13 hours from HCG injection: the picture of the Graafian follicles after13 hours from HCG injection and nearly after 5 hours of reaching lysosomal bodies its maximum size is that the entire follicular wall, more in the apical part, is *starting* to breakdown **(Morioka et al., 1989 and Schroeder and Talbot, 1998)**. The missing portions of the epithelial layer will expose beneath or uncover the orifices or the terminal ends of the communicating channels that developed from vacuolated lysosomal bodies **(Bjersing and Cajander, 1975 and Cajander and Bjersing, 1976)**. This porous picture and the dissolution of follicular apex that precede rupture may be maximized by the simultaneous local ovarian inflammatory reactions and edema **(Bjersing and Cajander, 1975; de Crespigny et al., 1981 and Espey, 1994)**. This leaky period in the follicular wall may extend about 20 hours, nearly from 13 hours from HCG injection up to the starting of follicular collapse. This period may constitute a window wherein the slowly oozing follicular fluid from follicles containing mature/maturing oocyte provides the communication or chemotactic signal to perceptive sperm. *Mathematically*, in our reported patient **(Nada et al., 2005)**, the estimated suggested time of fertilization inside the Graafian follicles is started between 9-13 hours before OPU, and we did the OPU after 34 hours from the HCG injection. So, we may strongly conclude that around 21 hours from the HCG injection, supported by the literatures **(de Crespigny et al., 1981; Schroeder and Talbot, 19982 and Morioka et al., 1989)** is the time during which the follicular walls are in the peak of breaking down process and may be porous enough to allow the passage of the sperm inside the follicles.

Rare phenomenon or normal but unnoticed event: the presence of the sperm inside Graafian follicle may be either a rare phenomenon or it may be just unnoticed regular but transient event.

If it is a rare phenomenon, it is hard to be explored further as it may be the result of an exceptional abnormal behavior or hyperactivation of the sperm and the reasons behind these changes may remain unknown. On the other hand, we may suggest that the ovary may be the normal site /or one of the normal sites of fertilization. But, the high incidence of male and tubal factors and other factors that prevent upward progression of sperm or impede their function or affect negatively on the pre-contact sperm-egg communication, as may frequently seen in IVF patients along with the short/transient time window for possible observation may explain the rarity of our finding. Also, abstinence for 2 or more days **(Cajander and Bjersing, 1976; de Crespigny et al., 1981and Eisenbach, 1999)** before OPU as generally instructed to those patients could be a restrictive factor making this observation so rare.

Last but not least, the field of *in-vitro* fertilization in humans has opened up unique window of opportunities to explore/investigate various early *in-vivo* events leading to fertilization, which were not amenable to study before. The conventional IVF and intracytoplasmic sperm injection couples with normal semen parameters (or even the intrauterine insemination couples) may form a suitable subject group; if counseled to have intercourse the day before OPU. The subjected group for investigation has to fulfill the criteria that the causes of their infertility are not related to factors that may prevent or impede the fertile sperm to reach the Graafian follicles with a capability to reach, recognize and fertilize

the egg. Selection of those couples may be difficult, and the ideal candidates may be the fertile couples preferably the highly fertile ones, but to find and convince them to participate in such investigation be the problem.

3. CONCLUSION

The sperm have the capabilities to search for the eggs, particularly the mature ones, even if they are in the Graafian follicles and before ovulation. The sperm may penetrate or may go inside the follicle through apertures or pores developed in its wall during ovulation process. There may be certain communication processes or languages between the two gametes, but the nature of this communication is not yet resolved.

Our documented finding of fertilization inside intact Graafian follicle needs further investigations; however, this may provide an *in-vivo* evidence of sperm capabilities to recognize the eggs, intact functional status of the sperm inside the Graafian follicles and also denotes that the follicular environment in mature follicles is conducive to fertilization. This may add help to approach and further explore the dilemmas of gamete recognition and possible sites of fertilization process.

REFERENCES

[1] Bjersing L, Cajander S. Ovulation and the role of the ovarian surface epithelium. Experientia. 1975; 15;31(5):605-8.

[2] Cajander S, Bjersing L.Further studies of the epithelium covering preovulatory rabbit follicles with special reference to lysosomal alterations. Cell Tissue Res. 1976 Jun 14;169(2):129-41.

[3] de Crespigny LC, O'Herlihy C, Robinson HP. Ultrasonic observation of the mechanism of human ovulation. Am J Obstet Gynecol. 1981; 15;139(6):636-9.

[4] Eisenbach M. Mammalian sperm chemotaxis and its association with capacitation. Dev Genet. 1999;25(2):87-94

[5] Eisenbach M, Tur-Kaspa I. Do human eggs attract spermatozoa? Bioessays. 1999; 21(3):203-10.

[6] Espey LL. Current status of the hypothesis that mammalian ovulation is comparable to an inflammatory reaction. Biol Reprod. 1994 Feb;50(2):233-8.

[7] Familiari G, Verlengia C, Nottola SA, Tripodi A, Hyttel P, Macchiarelli G, Motta PM. Ultrastructural features of bovine cumulus-corona cells surrounding oocytes, zygotes and early embryos. Reprod Fertil Dev. 1998;10(4):315-26.

[8] Gilula NB, Epstein ML, Beers WH. Cell-to-cell communication and ovulation. A study of the cumulus-oocyte complex. J Cell Biol. 1978 Jul;78(1):58-75.

[9] Granot I, Dekel N. Cell-to-cell communication in the ovarian follicle: developmental and hormonal regulation of the expression of connexin43. Hum Reprod. 1998; 13 Suppl 4:85-97.

[10] Hallatt JG. Primary ovarian pregnancy: a report of twenty-five cases. Am J Obstet Gynaecol 1982;143:55– 60.

[11] Jaiswal BS, Tur-Kaspa I, Dor J, Mashiach S, Eisenbach M. Human sperm chemotaxis: is progesterone a chemoattractant? Biol Reprod. 1999; 60(6):1314-9.

[12] Jeon BG, Moon JS, Kim KC, Lee HJ, Choe SY, Rho GJ. Follicular fluid enhances sperm attraction and its motility in human. J Assist Reprod Genet. 2001; 18(8):407-12.

[13] Kunz G, Beil D, Deiniger H, Einspanier A, Mall G and Leyendecker G. The uterine peristaltic pump. Normal and impeded sperm transport within the female genital tract. Adv Exp Med Biol. 1997; 424:267-77.

[14] Leon Speroff, Robert H. Glass and Nathan G. Kase, eds. Regulation of menstrual cycle. In: clinical Gynaecologic Endocrinology and Infertility. Fifth ed. Baltimore,Maryland,USA: William &Wilkins, 1994:183.

[15] Makler A, Reichler A, Stoller J, Feigin PD. A new model for investigating in real-time the existence of chemotaxis in human spermatozoa. Fertil Steril. 1992; 57(5):1066-74.

[16] Makler A, Stoller J, Reichler A, Blumenfeld Z, Yoffe N. Inability of human sperm to change their orientation in response to external chemical stimuli. Fertil Steril. 1995; 63(5):1077-82.

[17] Morioka N, Zhu C, Brannstrom M, Woessner JF, LeMaire WJ. Mechanism of mammalian ovulation. Prog Clin Biol Res. 1989;294:65-85.

[18] Nada ZM, Assaf NF and Dubey SK. Human sperm can fertilize ova inside Graafian follicle before ovulation. Fertil. Steril. 2005;38:457-61.

[19] Noujua-Huttunen S, Toumivaara L, Juntunen K, Tomاs C, Kauppila A, Martikainen H. Intrafollicular insemination for the treatment of infertility. Hum Reprod 1995;10:91–3.

[20] Ralt D, Goldenberg M, Fetterolf P, Thompson D, Dor J, Mashiach S, Garbers DL and Eisenbach M. Sperm attraction to a follicular factor(s) correlates with human egg fertilizability. Proc. Natl. Acad. Sci. U S A. 1991; 1:88(7):2840-4.

[21] Schroeder PC, Talbot P. Intrafollicular pressure decreases in hamster preovulatory follicles during smooth muscle cell contraction in vitro. J Exp Zool. 1982 Dec 30;224(3):417-26.

[22] Sun F, Bahat A, Gakamsky A, Girsh E, Katz N, Giojalas LC, Tur-Kaspa I. Eisenbach M. Human sperm chemotaxis: both the oocyte and its surrounding cumulus cells secrete sperm chemoattractants. Hum Reprod. 2005; 20(3):761-767.

[23] Tacconis P, Revelli A, Massobrio M, Battista La Sala G, Tesarik J.Chemotactic responsiveness of human spermatozoa to follicular fluid is enhanced by capacitation but is impaired in dyspermic semen. J Assist Reprod Genet. 2001; 18(1):36-44 .

[24] Villanueva-Diaz C, Arias-Martinez J, Bustos-Lopez H and Vadillo-Ortega F. Novel model for study of human sperm chemotaxis. Fertil. Steril. 1992; 58(2):392-5.

[25] Virutamasen P, Smitasiri Y, Fuchs AR. Intraovarian pressure changes during ovulation in rabbits. Fertil Steril. 1976 Feb;27(2):188-96.