10.5005/jp-journals-10006-1471

REVIEW ARTICLE

Empty Follicular Syndrome: Understanding Controversial Entity

[1]Sumesh D Choudhary, [2]Vineet Mishra

ABSTRACT

Empty follicular syndrome (EFS) is defined as the failure to retrieve oocytes from mature ovarian follicles after controlled ovarian hyperstimulation (COH) for *in vitro* fertilization (IVF). There are two types of EFS—genuine and false EFS. In genuine EFS, there is failure to retrieve oocytes from mature ovarian follicles after COH for IVF after apparently normal follicular development and steroidogenesis in the presence of optimum β-human chorionic gonadotropin (hCG) levels, whereas in false EFS, there is failure to retrieve oocytes in the presence of low β-hCG levels. Whatever may be the cause of EFS, these patients should be counseled regarding its possibility of recurrence and future poor prognosis. However, different IVF treatment methods in subsequent cycles could modulate the response with successful oocyte recovery in such cases.

Keywords: Genetic factor, Human chorionic gonadotropin, Oocyte retrieval, Ovarian follicle, Ovulation induction.

How to cite this article: Choudhary SD, Mishra V. Empty Follicular Syndrome: Understanding Controversial Entity. J South Asian Feder Obst Gynae 2017;9(2):119-123.

Source of support: Nil

Conflict of interest: None

Date of received: 1 January 2017

Date of acceptance: 11 February 2017

Date of publication: March 2017

INTRODUCTION

The nature of empty follicular syndrome (EFS) has been controversial since its description by Coulam et al[1] in 1986. The condition was defined as the failure to retrieve oocytes from mature ovarian follicles after controlled ovarian hyperstimulation (COH) for *in vitro* fertilization (IVF), even after meticulous aspiration and repeated flushing, despite apparently normal follicular development and estradiol (E2) levels.[2] The EFS is estimated to affect <1 to 7% patients undergoing IVF treatment.[3]

[1]Assistant Professor, [2]Professor

[1,2]Department of Obstetrics and Gynecology, Smt. G. R. Doshi & Smt. K. M. Mehta Institute of Kidney Diseases & Research Centre, Ahmedabad, Gujarat, India

Corresponding Author: Sumesh D Choudhary, Assistant Professor, Department of Obstetrics and Gynecology, Smt. G. R. Doshi & Smt. K. M. Mehta Institute of Kidney Diseases & Research Centre, Ahmedabad, Gujarat, India, Phone: +91-7922687031, e-mail: drsumeshchoudhary@gmail.cim

Although occurrence of EFS is rare, it is really a frustrating complication of IVF leading to cycle cancellation, which may cause substantial stress and anxiety for both patients and treating physicians. It is, therefore, very important to understand EFS.

In 2008, Stevenson and Lashen[4] categorized EFS into "false" or "genuine" according to β-human chorionic gonadotropin (hCG) levels on the day of oocyte retrieval. In their review, 33% cases were genuine EFS (G-EFS) and 67% were labeled as false EFS. The G-EFS was defined as failure to retrieve oocytes from mature ovarian follicles after COH for IVF after apparently normal follicular development and steroidogenesis in the presence of optimum β-hCG levels on the day of oocyte retrieval, and "false EFS" as failure to retrieve oocytes in the presence of low β-hCG due to an error in the administration of hCG or its bioavailability on the day of oocyte retrieval.

ETIOLOGY AND PATHOGENESIS

The underlying mechanism of EFS remains hypothetical. It has been suggested that it is not a syndrome, but rather a sporadic event that cannot be predicted by the pattern of ovarian response either sonographically or hormonally.[5] Many hypotheses have been put forward ranging from human error to pharmacologic problems. The underlying pathology is unknown; however, it has been earlier suggested that EFS might reflect dysfunctional folliculogenesis with an altered follicular steroid profile.[6] Others have interpreted EFS solely as a drug-related syndrome, resulting from an abnormality in the biological activity of some batches of hCG[7] or human error related to injections.[8] However, these hypotheses fail to explain the recurrence of the syndrome in certain patients.[9]

The underlying mechanism of EFS still remains unresolved. Dysfunctional folliculogenesis and genetic factors have been implicated in the etiology of EFS.[10,11] Molecular studies using transcriptional profiling showed that genes involved in apoptosis were differentially expressed in the granulosa cells from a patient with recurrent EFS, suggesting a role in the disappearance of the oocyte.[12] The luteinizing hormone/choriogonadotropin receptor (LHCGR) is a glycoprotein hormone receptor belonging to the G-protein coupled receptor family. Loss-of-function mutations of LHCGR lead to

Leydig cell hypoplasia and pseudohermaphroditism in males. It can lead to infertility in sisters of these affected males having the same biallelic LHCGR mutations.[13] Also, female mice lacking LHCGR were found to have no preovulatory follicles or corpora lutea. It was likely secondary to defective folliculogenesis and ovulation.[14] There is strong evidence that LHCGR p.N400S causes EFS and that the condition is inherited as a recessive genetic trait. The mutant allele cosegregates with EFS in the family, it is absent in ethnicity-matched controls, the critical amino acid residue is highly conserved, and earlier *in vitro* studies showed that a mutation at this residue results in reduced β-hCG binding to the receptor. From these results, it can be concluded that mutation of LHCGR leads to G-EFS, which cannot be resolved with repeated administration of β-hCG, because the receptor is impaired. All the above evidences suggest that G-EFS is present and that it might be the real cause of infertility in recurrent cases. Some reports propose an error in folliculogenesis or premature apoptosis of the oocytes that still continued follicular growth.[15]

Study of terminal follicular development suggests that, in rare cases, follicles may need longer exposure to β-hCG to undergo cumulus expansion and separate from the follicular wall.[16] It is worth noting that EFS has been reported with immature oocytes that were zona-free or that had a zona that was lacking in oocytes.[17] The LH-dependent cumulus expansion requires communication between the mural granulosa cells and the cumulus complex involving amphiregulin, epiregulin, and beta-cellulin.[18] These growth factors induce expression of prostaglandin synthase2, tumor necrosis factor alpha-induced protein, and hyaluronan synthase2, which, in turn, are necessary for cumulus expansion and subsequent oocyte release. It remains to be determined whether G-EFS is accompanied by altered expression of genes regulating cumulus expansion, but studies in mice support the possibility of such a mechanism.[19]

PREDICTION OF EFS

The EFS is a rare complication of IVF that cannot be anticipated before the ovum pickup procedure. Van Heusden et al[20] suggested that EFS is just due to mathematical coincidence. The oocyte recovery rate in natural cycles is approximately 80%. Based on the suggestion by Van Heusden et al,[20] the chance to recover one oocyte per follicle is similar in conventional IVF, but mathematical chance for failure to recover any oocyte is considerable. For example, in their report, 14 patients had 2 to 6 dominant follicles present before oocyte retrieval. Given this number of dominant follicles, the calculated chance for failure to recover at least one oocyte is 0.064 to 4%. Hence, in relation to the reported incidence of EFS, this mathematic coincidence should be considered. Based on the findings of Van Heusden et al,[20] the likelihood of failed oocyte retrieval due to chance alone for the cases of G-EFS was reported to be 0.001 and 0.00005%, for 7 and 9 follicles respectively [likelihood of failed oocyte retrieval = $(0.2)^n$; where n = number of follicles]. The prevalence of G-EFS they detected was substantially greater (>16-fold) than might be expected due to chance alone; therefore, they interpreted the findings to support the existence of G-EFS. Mesen et al[21] had observed that G-EFS can be present when >20 follicles are present on ultrasound, strongly suggesting that chance alone is not a satisfactory explanation for the phenomenon. Ovarian aging has also been suggested to have a significant role in G-EFS.[12] Other investigators have considered low ovarian reserve as the cause of recurrence of EFS in subsequent treatments.[22] Genetic causes of EFS have also been proposed and reported on a possible inherited condition of EFS with moderate sensorineural deafness. Any alteration that changes the transient and sequential expression of epidermal growth factor in family members might affect the oocyte growth in follicles, owing to impaired cumulus expansion and oocyte release.[23] Recurrent EFSs occurred in 15.8% of subsequent cycles.[24]

Prevention

Ovarian reserve assessment in an assisted reproductive treatment setting has improved considerably in the past few years, especially when the target is to evaluate ovarian response to COH. Both antimullerian hormone and antral follicle count have been shown to be reliable, reproducible, and to have high prediction potential for ovarian reserve appraisal. To prevent EFS, it is important to select patient with optimum ovarian reserve. Final follicular maturation in gonadotropin-releasing hormone (GnRH) agonist protocol is triggered as soon as the majority of the leading preovulatory follicles have reached a diameter of 17 mm, preferably with a ratio of E2 level to number of leading preovulatory follicles of >14 mm calculated to be lower than 100 pg/mL. The GnRH antagonist should be given on cycle day 6 (when the serum E2 level was >1,000 pmol/L) and continued until the day of β-hCG administration. This will help to avoid premature trigger and prevent from aspirating immature oocyte or only follicular fluid (FF).

The etiology of failed injections is challenging; it may result from human error in either mixing or administering the medication or rapid clearance of the medication secondary to loss of sialylated side chains.[25] The best proposal for potential preventative measure against iatrogenic EFS is through the assessment of posttrigger serum β-hCG level. Human chorionic gonadotropin levels

lower than 10 IU/L, 36 hours after the presumed injection, indicate a missed administration or an alteration of the *in vivo* biological activity of β-hCG.[26] The absence of cumulus cells into the FF may also help in diagnosis.[27] It is important to check β-hCG levels and also serum LH and progesterone (P) levels to ensure appropriate timing of the second oocyte retrieval. The threshold amplitude to define an LH surge is still a matter of debate, but levels more than 10 IU/L are commonly reported.[28] The second puncture should be planned 24 hours later of LH peak (LH>10 IU/L and P<1.5 ng/mL). Patients requiring a second β-hCG bolus before retrieval can be detected, and their cycles are salvaged. One ovary to be aspirated, if no oocyte is retrieved, and checked for premature ovulation and when and how β-hCG was administered. If no administrative fault, then measure the β-hCG, if less (<40 mIU/mL), plan for rescue protocol using (10,000 u) β-hCG from different batch or r-hCG; plan retrieval after 36 hours. If β-hCG is normal, try to retrieve oocyte from second ovary. If no oocyte is retrieved, repeat next cycle with gentle stimulation protocol and r-hCG or recombinant LH. The second option is use of GnRH agonist in antagonist protocol cycle to trigger endogenous LH surge or the same protocol using a different batch of β-hCG.

Given the rarity of failed hCG injection in IVF populations, such interventions will have only a very small impact on overall clinical success rates. However, careful attention to every detail can greatly benefit individual patients.[29] When examining P levels at the time of β-hCG trigger, previous studies have suggested discriminatory levels from 1.2 to 4.0 ng/mL as a critical threshold in predicting successful pregnancy.[30] As an elevated P does not appear to affect the number of mature oocytes retrieved or fertilization rates, it effects the implantation rate.[31] Despite the recent report of successful oocyte retrieval using r-hCG in a similar case, there is a lack of theoretical rationale to explain how this approach would work differently with the already-proven bioavailability of u-hCG administered.[32] By increasing the time of exposure to β-hCG, no consistent differences in oocyte yield, maturity, fertilization, or embryo quality were detected. However, as age increases, significant trends toward improved implantation rates, clinical pregnancy, and live births were detected by extending exposure to hCG for > 36.5 hours. It may be beneficial in patients aged > 40 years.[33]

MANAGEMENT

The effect of the stimulation protocol on the risk of EFS is not known. Some have postulated that EFS is a drug-related problem rather than a clinical dysfunction,[34] whereas others suggested that the occurrence of EFS in IVF can be attributed to a failure in (a) accurate timing of induction of final oocyte maturation, (b) properly scheduled ovarian hyperstimulation, or (c) instruction of patients and doctors.[35]

However, by differentiating between the false and genuine types, these suggestions become less relevant. A second, rescue dose of β-hCG in the setting of false EFS was first proposed by Ndukwe et al[29], and this has persisted as the consensus solution in the literature since that time. Although isolated case reports have described pregnancies from this approach, the largest single case series shows a limited prognosis.[36]

Live-birth pregnancies are a realistic possibility after administration of a rescue course of β-hCG and repeat oocyte retrieval in the setting of false EFS.[37] The risk of recurrence of EFS is 20%, the risk being higher with advancing age: 24% recurrence rate for age 35 to 39 years and 57% for age over 40 years.[2] The failure to retrieve the oocyte during the follicle phase may be attributed to EFS, but it cannot be excluded completely that not enough time was allowed before retrieval. In G-EFS, dysfunction of the folliculogenesis seems to be the most plausible etiology.[6] In fact, when they analyzed the etiology of infertility in the IVF patients group, they found a higher proportion of polycystic ovarian syndrome with low response and a lower percentage of unexplained infertility, which supports the concept of dysfunctional folliculogenesis.

As previously mentioned, in contrast to hCG triggering, the action of a bolus of GnRH agonist is indirect via the endogenous release of LH and follicle stimulating hormone (FSH) from the pituitary after binding to and activation of the GnRH receptor.[38] As the pituitary is the target organ for GnRH agonist, one might assume that under temporary or permanent dysfunctions of the pituitary, a sufficient flare-up effect will not be achieved, resulting in a deficient final follicular maturation and EFS.

An example of this is the hypogonadotropic/hypogonadal patient (World Health Organization type I) who is characterized by endogenous levels of LH and FSH below 1.2 IU/L. The GnRH agonist triggering in this type of patient will invariably result in EFS due to the induction of an insufficient surge of gonadotropins. Patients who could be hypothesized to develop EFS after GnRH agonist triggering are patients with a GnRH receptor polymorphism,[39] necessitating a higher dose of GnRH agonist to activate the receptor in line with the FSH receptor polymorphism (Ser/680 FSH-R).[40] The same would account for patients with an LH receptor polymorphism.[41] Patients with a variant LH beta gene polymorphism, specifically in the homozygous form, resulting in a less bioactive LH molecule[42,43] might be at risk to have a blunted response after GnRH agonist triggers the incidence of EFS seems to be similar after GnRH agonist and β-hCG triggering. As in EFS cases seen after

β-hCG, the exact reason for failure after GnRH agonist triggering remains uncertain. If the oocytes are present but failed to mature in the follicles during stimulation, it may be more effective to remove the immature oocytes and apply the maturation process *in vitro*. Although the β-hCG was negative, this case could lead to an alternative approach to G-EFS.[44] Most cases of EFS after either β-hCG or GnRH agonist trigger are related to human error, and, thus, a meticulous counseling and instruction of the patient prior to oocyte retrieval is of outmost importance.

CONCLUSION

Ovarian follicles of patients with so-called EFS may not actually be devoid of viable oocytes. The problem seems to be that of inadequate preovulatory follicular changes arising from either poor bioavailability of LH or β-hCG or too short an interval between the onset of these changes and follicular aspiration. Premature lutenization due to a premature LH surge and high P levels on the day of β-hCG injection can also affect the oocyte recovery. The EFS does not predict a reduced fertility potential in future cycles. Nevertheless, whatever the cause of EFS, these patients should be counseled regarding its possibility of recurrence and future poor prognosis. However, different IVF treatment methods in subsequent cycles could modulate the response with successful oocyte recovery in such cases.

REFERENCES

1. Coulam CB, Bustillo M, Schulman JD. Empty follicle syndrome. Fertil Steril 1986 Dec;46(6):1153-1155.
2. Zreik TG, Garcia-Velasco JA, Vergara TM, Arici A, Olive D, Jones EF. Empty follicle syndrome: evidence for recurrence. Hum Reprod 2000 May;15(6):999-1002.
3. Bartfai G, Feinman M, Barad G. Empty follicle syndrome. Fertil Steril 1987 Jun;47(6):1040-1042.
4. Stevenson TL, Lashen H. Empty follicle syndrome: the reality of a controversial syndrome, a systematic review. Fertil Steril 2008 Sep;90(3):691-698.
5. Ben-Sholmo I, Schiff E, Levran D, Ben-Rafael Z, Mashiach S, Dor J. Failure of oocytes retrieval during in vitro fertilization: a sporadic event rather than a syndrome. Fertil Steril 1991 Feb;55(2):324-327.
6. Tsuiki A, Rose BI, Hung TT. Steroid profiles of follicular fluids from a patient with the empty follicle syndrome. Fertil Steril 1988 Jan;49(1):104-107.
7. Zegers-Hochschild F, Fernandez E, Mackenna A, Fabres C, Altieri E, Lopez T. The empty follicle syndrome: a pharmaceutical industry syndrome. Hum Reprod 1995 Sep;10(9):2262-2265.
8. Snaifer E, Hugues JN, Poncelet C, Sifer C, Pasquier M, Cedrin-Durnerin I. "Empty follicle syndrome" after human error: pregnancy obtained after repeated oocyte retrieval in a gonadotropin-releasing hormone antagonist cycle. Fertil Steril 2008 Sep;90(3):850,e13-e15.
9. La Sala G, Ghirardini G, Cantarelli M, Dotti C, Cavalieri S, Torelli MG. Recurrent empty follicle syndrome. Hum Reprod 1991;6:651-652.
10. Ashkenazi J, Feldberg D, Shelef M, Dicker D, Goldman JA. Empty follicle syndrome: an entity in the etiology of infertility of unknown origin, or a phenomenon associated with purified follicle-stimulating hormone therapy? Fertil Steril 1987 Jul;48(1):152-154.
11. Vujisic S, Stipoljev F, Bauman R, Dmitrovic R, Jezek D. Pericentric inversion of chromosome 2 in a patient with the empty follicle syndrome: case report. Hum Reprod 2005 Sep;20(9):2552-2555.
12. Inan MS, Al-Hassan S, Ozand P, Coskun S. Transcriptional profiling of granulos cells from a patient with recurrent empty follicle syndrome. Reprod Biomed Online 2006 Oct;13(4): 481-491.
13. Ashkenazi H, Cao X, Matola S, Popliker M, Conti M, Tsafrifi A. Epidermal growth factor family mediators of the ovulatory response. Endocrinology 2005 Jan;146(1):77-84.
14. Zhang FP, Poutanen M, Wilbertz J, Huhtaniemi I. Normal prenatal but arrested postnatal sexual development of luteinizing hormone receptor knockout (LuRKO) mice. Mol Endocrinol 2001 Jan;15(1):172-183.
15. Desai N, Austin C, AbdelHafez F, Goldfarb J, Falcone T. Evidence of "genuine empty follicles" in follicular aspirate: a case report. Hum Reprod 2009 May;24(5):1171-1175.
16. Meniru GI, Craft IL. Evidence from a salvaged treatment cycle supports an aetiology for the empty follicle syndrome that is related to terminal follicular developmental events. Hum Reprod 1997 Aug;12(11):2385-2387.
17. Duru NK, Cincik M, Dede M, Hasimi A, Baser I. Retrieval of zona-free immature oocytes in a woman with recurrent empty follicle syndrome: a case report. J Reprod Med 2007 Sep;52(9):858-863.
18. Zamah AM, Hsieh M, Chen J, Vigne JL, Rosen MP, Cedars MI, Conti M. Human oocyte maturation is dependent on LH-stimulated accumulation of the epidermal growth factor-like growth factor, amphiregulin. Hum Reprod 2010 Oct;25(10):2569-2578.
19. Hsieh M, Lee D, Panigone S, Horner K, Chen R, Theologis A, Lee DC, Threadgill DW, Conti M. Luteinizing hormone-dependent activation of the epidermal growth factor network is essential for ovulation. Mol Cell Biol 2007 Mar;27(5): 1914-1924.
20. Van Heusden AM, van Santbrink EJ, de Jong D, Schipper I. The empty follicle syndrome is dead! Fertil Steril 2008 Mar;89(3):746.
21. Mesen TB, Yu B, Richter KS, Widra E, DeCherney AH, Segars JH. The prevalence of genuine empty follicle syndrome. Fertil Steril 2011 Dec;96(6):1375-1377.
22. Younis JS, Skournik A, Radin O, Haddad S, Bar-Ami S, Ben-Ami M. Poor oocyte retrieval is a manifestation of low ovarian reserve. Fertil Steril 2005 Feb;83(2):504-507.
23. Onalan G, Pabuçcu R, Onalan R, Ceylaner S, Selam B. Empty follicle syndrome in two sisters with three cycles: case report. Hum Reprod 2003 Sep;18(9):1864-1867.
24. Baum M, Machtnger R, Yerushalmi GM, Maman E, Seidman DS, Dor J, Hourvitz A. Recurrence of empty follicle syndrome with stimulated IVF cycles. Gynecol Endocrinol 2012 Apr;28(4):293-295.
25. Quintans CJ, Donaldson MJ, Blanco LA, Pasqualini RS. Empty follicle syndrome due to human errors: its occurrence in an *in-vitro* fertilization programme. Hum Reprod 1998 Oct;13(10):2703-2705.

26. Ndukwe G, Thornton S, Fishel S, Dowell K, al-Hassan S, Hunter A. Predicting empty follicle syndrome. Fertil Steril 1996 Nov;66(5):845-847.
27. Abdalla IH, Ah-Moye M, Brisden P, Howe DL, Okonofus F, Craft I. The effect of the dose of human chorionic gonadotropin and the type of gonadotropin stimulation on oocyte recovery rates in an *in vitro* fertilization program. Fertil Steril 1987 Dec;48(6):958-963.
28. Lok F, Pritchard J, Lashen H. Successful treatment of empty follicle syndrome by triggering endogenous LH surge using GnRH agonist in an antagonist down-regulated. Hum Reprod 2003 Oct;18(10):2079-2081.
29. Ndukwe G, Thornton S, Fishel S, Dowell K, Aloum M, Green S. "Curing" empty follicle syndrome. Hum Reprod 1997 Jan;12(1):21-23.
30. Bosch E, Labarta E, Crespo J, Simon C, Remohi J, Jenkins J, Pellicer A. Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles for *in vitro* fertilization: analysis of over 4000 cycles. Hum Reprod 2010 Aug;25(8):2092-3100.
31. Nayak S, Ochalski ME, Fu B, Wakim KM, Chu TJ, Dong X, Wakim AN. Progesterone level at oocyte retrieval predicts *in vitro* fertilization success in a short-antagonist protocol: a prospective cohort study. Fertil Steril 2014 Mar;101(3): 676-682.
32. Peñarrubia J, Balasch J, Fábregues F, Creus M, Cívico S, Vanrell JA. Recurrent empty follicle syndrome successfully treated with recombinant human chorionic gonadotrophin. Hum Reprod 1999 Jul;14(7):1703-1706.
33. Reichman DE, Missmer SA, Berry KF, Ginsburg ES, Racowsky C. Effect of time between human chorionic gonadotropin injection and egg retrieval is age dependent. Fertil Steril 2011 May;95(6):1990-1995.
34. Ubaldi F, Nagy Z, Janssenwillen C, Smitz J, Van Steirteghem A, Devroey P. Ovulation by repeated hCG in "empty follicle syndrome" yields a twin clinical pregnancy. Hum Reprod 1997 Mar;12(3):454-456.
35. Aktas M, Beckers N, Van Inzem WG, Verhoeff A, de Jong D. Oocytes in the empty follicle: a controversial syndrome. Fertil Steril 2005 Dec;84(6):1643-1648.
36. Papier S, Lipowicz R, De Vincentiis S, Nodar F, Olmedo SB, Acosta A. Pregnancy obtained by the transfer of frozen-thawed embryos originating from a rescued empty follicle syndrome cycle. Fertil Steril 2000 Sep;74(3):603-604.
37. Doyle JO, Attaman JA, Styer AK, Sabatini ME, Petrozza JC, Toth TL. Rescue human chorionic gonadotropin for false empty follicle syndrome: optimism for successful pregnancy outcome. Fertil Steril 2012 Aug;98(2):450-452.
38. Gonen Y, Balakier H, Powell W, Casper RF. Use of gonadotropin releasing hormone agonist to trigger follicular maturation for *in vitro* fertilization. J Clin Endocrinol Metab 1990 Oct;71(4):918-922.
39. Beranova M, Oliveira LM, Bédécarrats GY, Schipani E, Vallejo M, Ammini AC, Quintos JB, Hall JE, Martin KA, Hayes FJ, et al. Prevalence, phenotypic spectrum, and modes of inheritance of gonadotropin-releasing hormone receptor mutations in idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab 2001 Apr;86(4):1580-1588.
40. Perez Mayorga M, Gromoll J, Behre HM, Gassner C, Nieschlag E, Simoni M. Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. J Clin Endocrinol Metab 2000 Sep;85(9):3365-3369.
41. Piersma D, Verhoef-Post M, Berns EM, Themmen AP. LH receptor gene mutations and polymorphisms: an overview. Mol Cell Endocrinol 2007 Jan;260-262:282-286.
42. Alviggi C, Clarizia R, Pettersson K, Mollo A, Humaidan P, Strina I, Coppola M, Ranieri A, D'Uva M, De Placido G. Suboptimal response to GnRHa long protocol is associated with a common LH polymorphism. Reprod Biomed Online 2009 Jan;18(1):9-14.
43. Pettersson KS, Söderholm JR. Individual differences in lutropin immuno reactivity revealed by monoclonal antibodies. Clin Chem 1991 Mar;37(3):333-340.
44. Hardy K, Wright CS, Franks S, Winston RM. *In vitro* maturation of oocytes. Br Med Bull 2000;56(3):588-602.