OPEN ACCESS Freely available online

Journal of Fertilization: In vitro - IVF- Worldwide, Reproductive Medicine, Genetics & Stem Cell Biology

Case Report

Priming with a GnRH Agonist Before Immature Oocyte Retrieval may Improve Maturity of Oocytes and Outcome in *In Vitro* Maturation (IVM) Cycle: A Case Report

Anna Smirnova[1*], Margarita Anshina[1], Einat Shalom Paz[2,3], Adrian Ellenbogen[3]

[1]*Department of Endocrinology, Russian University of People's Friendship, Moscow, Russia;*[2]*HillelYaffe Medical Center, Hadera, Israel;* [3]*Rappaport School of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*

ABSTRACT

In the last several years the concept of using a gonadotropin releasing hormone agonist (GnRH-a) for triggering ovulation in patients treated by an antagonist protocol for IVF became almost a routine clinical practice. It may promote oocyte nuclear maturation, resumption of meiosis and cumulus expansion. Several studies reported the retrieval of more mature oocytes after GnRH-a, therefore it seems that this attempt could be beneficial in an *in vitro* maturation (IVM) oocyte cycle performed for fertility preservation in patients with malignancies but possible for other indication as polycystic ovarian syndrome patients. We presented a case of a patient needed fertility preservation that underwent 3 IVM cycles priming ovulation with a GnRH-a. Twelve oocytes were obtained, all of them matured 4.5 hours after incubation in maturation media. Fertilization rate after ICSI was 10/12 (83%). Six good quality embryos were vitrified. It seems that triggering with a GnRH-a instead of hCG in an IVM cycle could be beneficial in terms of obtaining high grade embryos and possible pregnancy.

Keywords: GnRH aganist priming; *In vitro* maturation; Oocyte; IVF

INTRODUCTION

The conception of *in vitro* maturation of oocytes (IVM) was primarily published in literature in 1935 [1]. Experiments described in which ova, taken from tubes at various interval after fertile matting, were cultured in vitro. Thirty four years later, it was proposed the option to use IVM, rather than protocols of hormonal stimulation currently in use, for *in vitro* fertilization treatments [2]. The authors mentioned that "it may be possible to recover immature oocytes from several antral follicles, excluding the dominant preovulatory follicle, and mature them in proper culture". A successful IVM cycle in humans beings using immature oocytes retrieved from antral follicles was reported later [3], followed by the first delivery from *in vitro* maturation of oocytes recovered from untreated polycystic ovarian patients [4].

PCOs patients had become the first natural candidates for IVM treatment as early as 1994 [4]. In more recent years, the range of clinical application of IVM has been extended to women with physiologically normal ovaries (usually defined as normovulatory) having appropriate characteristics [5] and lately, IVM has been also emphasized as an additional opportunity for female germ cell preservation in women suffering from cancer [6-9]. Novel radio and chemotherapies treatments can significantly improve the prognosis of these patients [10], but unfortunately imply major effects on ovarian function, often leading to premature ovarian failure. Technically, immature oocytes collected from antral follicles in the absence of gonadotropin administration may be cryopreserved before or after maturation *in vitro* [11]. Therefore, IVM may represent a suitable opportunity for recovery of oocytes intended to cryopreservation in cases in which tumor estrogen-sensitivity and/or urgency to commence therapy opposed a full controlled ovarian stimulation management [12].

It is nowadays a common practice in many IVF units to perform triggering with human chorionic gonadotropin (hCG) before oocyte retrieval in an IVM cycle and extension the period time of egg retrieval from 35-36 hours (routinely administrated in IVF

Correspondence to: Smirnova A, Department of Endocrinology of Postgraduated Medical Faculty of Russian University of People's Friendship, Moscow, Russia, E-mail: a-smirnova@mail.ru

Received: February 05, 2021; **Accepted:** February 19, 2021; **Published:** February 26, 2021

Citation: Smirnova A, Anshina M, Paz ES, Ellenbogen A (2021) Priming with a GnRH agonist before Immature Oocyte Retrieval may Improve Maturity of Oocytes and Outcome in In Vitro Maturation (IVM) Cycle: A Case Report. J Fertil In vitro IVF Worldw Reprod Med Genet Stem Cell Biol. 9:229.

cycles) to 38 hours from hCG administration, that promotes GV oocytes to reach MI stage and increases the maturation rate of immature oocytes in *in vitro* setting [13]. However, different studies reported that implantation and pregnancy rates after IVM were lower compared with traditional IVF and varied from 5%-22% and 8%-40%, accordingly [14,15]. It is possible that in an IVM cycle, MII oocytes obtained after maturation in proper medium, even though present nuclear maturation that progress normally, the cytoplasm maturation is delayed, resulting in a poorer embryonic development, implantation and pregnancy rates [16]. Human chorionic gonadotropin has been the gold standard for ovulation induction as a surrogate for the mid-cycle LH surge forseveral decades. In the last several years the concept of using a GnRH-a for triggering ovulation was reintroduced in patients treated by an antagonist protocol for IVF. Interestingly, it was suggested that it may promote oocyte nuclear maturation, i.e. resumption of meiosis [17,18], and cumulus expansion [19,20]. Several studies reported the retrieval of more mature oocytes after GnRH-a trigger, which could be an effect of a more physiological surge including an FSH surge as well as an LH surge induced by the GnRh-a [21-23]. Recently it was suggested that triggering final oocyte maturation in IVF cycles with GnRH-a versus hCG in breast cancer patients undergoing fertility preservation could be beneficial, as evidenced by the number of mature oocytes and cryopreserved embryos [24,25]. Therefore it seems that this approach could be beneficial in an *in vitro* maturation oocyte cycle performed for fertility preservation in patients with malignancies.

CASE REPORT

A 38 years old woman was referred to IVF & Reproductive Genetics Center, Moscow, for fertility preservation. She was married for 8 years, has been never pregnant before and used barrier contraception. She had regular menstrual periods 25-28 days. Her past history revealed that she underwent laparoscopy and unilateral adnexectomy due to a large papillary mucinous cystadenoma 10 cm in diameter. Ten years later a new cyst, 4 cm in diameter was diagnosed on the contralateral ovary and laparotomy with partial ovarian resection was performed. Histological examination revealed again mucinous cystadenoma. On admission, hormonal profile on day 3 of cycle was: AMH- 0.35 ng/ml, FSH- 6.9 IU/l. Vaginal ultrasound examination (U/S) on day 7 of her cycle showed a normal size uterus with a small subserosal fibroid of 15x10 mm on posterior wall. The size of left ovary was 34x23x22 mm containing a cyst of 18x16 mm. and 5 antral follicles from 3 to 8 mm in diameter. Her 30 years old partner had normal sperm parameters. In accordance with Russian legislation, ovarian stimulation in women with ovarian tumors (even small), is prohibited.Therefore, a natural cycle IVF was commenced. After routine follow up, on day 10 of cycle, a dominant follicle of 16 mm in diameter was found by U/S examination. The endometrium thickness was 8.4 mm. Final follicle maturation was triggered with triptorelin (Decapeptyl, Ferring GmbH, Kiel, Germany) 0.2 mg subcutaneously administered on the same day. Transvaginal U/S-guided follicle aspiration was performed 31 hours after GnRH-a injection when the dominant follicle was 21 mm in diameter, but no oocyte was obtained. In order to improve results and obtaining oocytes without stimulating the ovaries, we decided to carry on with *in vitro* maturation cycle. Totally three IVM attempts were performed.

First IVM cycle

Ultrasound monitoring was started on day 6 of the cycle. Endometrial thickness was 5 mm and three antral follicles of 7.5 mm, 8.5 mm and 6 mm in diameter were visualized. On day 8 of cycles, the follicles were 8 mm, 9.5 mm and 7 mm and triptorelin 0.2 mg subcutaneously (SC) was administered for triggering oocytes maturation. Transvaginal U/S-guided follicle aspiration was performed 39 hours later by 19G/17G single lumen needle (Swemed Sense, Vitrolife, Göteborg, Sweden), using a reduced aspiration pressure of 7.5kPa. Follicular fluid was collected without flushing into 50 ml dishes with Oocyte Washing Medium (SAGE IVM media kit, Sage, Cooper Surgical Company, USA). Totally 3 oocyte-cumulus complexes (COCs) were obtained. All COCs were cultured in maturation medium (SAGE IVM media kit, Cooper Surgical Company, USA) supplemented with FSH+LH (Merional, IBSA Institute Biochimique S.A., Switzerland) for a final concentration of 75 mIU/ml. Retrieved oocytes were stripped, found in the MII stage and fertilized by ICSI 6 hours after aspiration as described previously [26]. All oocytes were fertilized 18 hours after ICSI. Two top quality embryos (8-cells grade 1) and one good quality embryo (8-cells grade 2) were vitrified 72 hours after fertilization. No luteal phase support was given to the patient.

Second IVM cycle

Eight days after commencement of menstrual cycle, vaginal U/S revealed an endometrium thickness of 5 mm and five antral follicles 8 mm, 8.5 mm, 7 mm, 5 mm and 5 mm in diameter were visualized on the left ovary. Two days later a 6.5 mm endometrium thickness and five antral follicles of 10 mm, 10.5 mm, 8 mm, 6 mm and 6 mm in diameter were demonstrated. Triptorelin 0.2 mg SC was administrated on the same day. Ovum pick up was performed 38.5 hours later. Six COCs were obtained. All of them were cultured in maturation media, stripped and fertilized by ICSI 4.5 hoursafter pick up. Normal fertilization occurred in 5 oocytes, the sixth one developed only one pronucleus. Only one top quality embryo (10-cells grade 1) was vitrified 72 hours after ICSI. Other 4 embryos were cultured till day 6, two poor qualityblastocysts (grade CC) were obtained and discarded.

Third IVM attempt

On the next menstrual cycle, ultrasound scan performed on day 10 and revealed an endometrium thickness of 7 mm and four antral follicles of 10 mm, 8 mm, 6 mm and 5 mm in diameter. Transvaginal follicle aspiration was performed 39 hours after triptorelin 0.2 mg SC injection as described above. Three COCs were obtained. All COCs were cultured in maturation media for 5 hours, stripped, found on MII stage and fertilized by ICSI. Two of them developed 2PN, 18 hours after fertilization. Two good quality embryos were vitrified on day 3. In summary, 12 COCs were obtained following IVM of small antral follicle aspiration. All oocytes were found matured (MII) about 4.5 hours after incubation in maturation media. Fertilization rate after ICSI was 10/12 (83%) and cleavage rate was 8/12 (67%).

Totally six good quality embryos were vitrified on day 3. After performing cystectomy in order to rule out malignancy, frozen embryo transfer will be planned. The characteristic of patient is shown in Table 1.

	COCs Obtained	Embryos Identified
First IVM	3	1
Second IVM	6	2
Third IVM	12	2

Table 1. Patient Characteristics

DISCUSSION

The emerging technology of IVM in oocyte retrieval has recently become another option for fertility preservation. This procedure can be done without hormonal stimulation and within a short time frame; oocytes being collected during the follicular phase [7,10] or even luteal phase treatment with a reasonable number of harvested oocytes [8]. Therefore, in cases of patients with malignancies, especially in which hormonal treatment is contraindicated and those who must start chemotherapy without delay, IVM might be a preffered option to preserve fertility. The flare-up effect of GnRH-a is used widely for induction of final oocyte maturation in traditional IVF antagonist protocols, mainly in order to avoid ovarian hyperstimulation syndrome which usually associated with administration of hCG [22]. Oktay et al. [24] reported for the first time the use of a GnRH analog as an oocyte maturation trigger in women with breast cancer undergoing IVF and found that the number of oocytes retrieved, maturation and fertilization rates and the number of 2PN embryos were significantly higher as compared with hCG trigger. Similar results were reported in breast cancer patients undergoing urgent fertility preservation [25-27]. Recently, Dahan et al. [28] described GnRH-a triggering in a variation on the modified natural IVF cycle in a patient with polycystic ovary syndrome. In this approach, follicles were stimulated with gonadotropins for three to five days when they were small and triggering of ovulation with a GnRH-a when the largest follicles were 10 to 12 mm in diameter. Many immature oocytes were retrieved, matured *in vitro* and subsequently developed to form blastocysts that resulted in a live birth. The rationale of triggering with a GnRH-a instead of hCG, mainly in a IVM cycle is the induction of FSH surge comparable to the surge of the natural cycle. This surge may promotes formation of LH receptors on granulose cells enhancing LH activity, induced plasminogen activator activity causing dissociation of oocytes from follicle wall (therefore more oocytes–immature-could be obtained), maintaining the opening of gap junction between cumulus cells and oocyte (enhancing oocyte maturation) [29-32], cumulus expansion [2,20] and possible promoting cytoplasmic maturation thought to be delayed in IVM cycle triggering with hCG before oocyte retrieval. In addition to endogenous LH and FSH surges that lead to oocyte maturation, it has been described that GnRH has receptors on granulosa cells that may play a role in regulating ovulation [33].

There are evidences that the release of LH and FSH after a single administration of GnRH-a in an IVF cycle is able to complete the final stage of follicular maturation, resulting in retrieval of fertlizable oocytes, with normal embryo development end pregnancy [34]. Therefore, the combined action of GNRH-a, FSH and LH may have a beneficial effect on oocyte maturation mainly in an IVM cycle. In our 3 IVM cycles, 12 oocytes were obatained. Interestingly, all oocytes (100%) were found mature (MII) after 4.5-5 hours culture in maturation media. This is in contrast with results of 1224 oocytes retrieved in IVM cycles after hCG triggering were only 15.6% were found mature up to six hours after retrieval vs. 64.9% that matured *in vitro* after 6-48 hours [35]. Indeed it could be suggested that in our case the mature eggs obtained short time after retrieval give rise to high grade embryos with possible high potential to implant, are nor "really" IVM oocytes from the semantic point of view. However, the most important point is the results in obtaining six high quality embryos that were vitrified.

CONCLUSION

To the best of our knowledge, this case report is one of the few reports in the literature of priming with GnRH-a before ocyte retrieval in an IVM protocol. In the recent years the results of IVM are continuously improving. Triggering ovulation with a GnRH-a, as described above, may carry an additional benefit. It seems that such attempt, instead of hCG, in an IVM cycle performed for fertility preservation could be valuable in terms of obtaining higher grade embryos, possible more synchronically nuclear and cytoplasmatic maturation and increasing the chance in obtaining a future pregnancy with minimal side effects toward the patients. A prospecting study using this approach is suggested in IVM cycles performed for other indications, as polycystic ovarian syndrome, avoiding the risk of ovarian hyper stimulation.

REFERENCES

1. Pincus G, Enzmann EV. The comparative behavior of mammalian eggs in vivo and in vitro I. The activation of ovarian eggs. J EXP Med. 1935;62(5):665-675.
2. Eppig JJ, Schroeder AC. Capacity of mouse oocytes from preantral follicles to undergo embryogenesis and development to live young after growth, maturation, and fertilization in vitro. Biol. Reprod. 1989;41(2):268-276.
3. Cha KY, Koo JJ, Ko JJ, Choi DH, Han SY, Yoon TK. Pregnancy after in vitro fertilization of human follicular oocytes collected from nonstimulated cycles, their culture in vitro and their transfer in a donor oocyte program. Fertil Steril. 1991;55(1):109–113.
4. Trounson A, Wood C, Kausche A. In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. Fertil Steril. 1994;62(2):353–362.
5. Fadini R, Canto MBD, Renzini MM, Brambillasca F, Comi R, Fumagalli D, et al. Effect of different gonadotrophin priming on IVM of oocytes from women with normal ovaries: A prospective randomized study. Reprod Biomed Online. 2009;19(3):343–351.
6. Gidoni YS, Takefman J, Holzer HE, Elizur SE, Son WY, Chian RC, et al. Cryopreservation of a mother's oocytes for possible future use by her daughter with Turner syndrome: Case report. Fertil Steril. 2008;90:9-12.
7. Huang JY, Chian RC, Gilbert L, Fleiszer D, Holzer H, Dermitas E, et al. Retrieval of immature oocytes from unstimulated ovaries followed

by in vitro maturation and vitrification: A novel strategy of fertility preservation for breast cancer patients. Am J Surg. 2010;200(1):177–183.

8. Maman E, Meirow D, Brengauz M, Raanani H, Dor J, Hourvitz A. Luteal phase oocyte retrieval and in vitro maturation is an optional procedure for urgent fertility preservation. Fertil Steril. 2014;95(1):64–67.
9. Shalom-Paz E, Almog B, Shehata F, Huang J, Holzer H, Chian RC, et al. Fertility preservation for breast-cancer patients using IVM followed by oocyte or embryo vitrification. Reprod Biomed Online. 2010;21(4): 566–571.
10. Mertens AC, Yasui Y, Neglia JP, Potter JD, Nesbit ME, Smithson KRWA, et al. Late mortality experience in five-year survivors of childhood and adolescent cancer: The childhood cancer survivor study. J Clin Oncol. 2001;19(13):3163-3172.
11. Cao Y, Xing Q, Zhang ZG, Wei ZL , Zhou P, Cong L. Cryopreservation of immature and in-vitro matured human oocytes by vitrification. Reprod Biomed Online. 2009;19(3):369-373.
12. Wallace WHB. Oncofertility and preservation of reproductive capacity in children and young adults. Cancer. 2011;117(S10):2301-2310.
13. Son WY, Chung JT, Chian RC, Herrero B, Demirtas E, Elizur S, et al. A 38 h interval between hCG priming and oocyte retrieval increases in vivo and in vitro oocyte maturation rate in programmed IVM cycles. Hum Reprod. 2008;23(9):2010-2016.
14. Mikkelsen AL, Smith SD, Lindenberg S. In-vitro maturation of human oocytes from regularly menstruating women may be successful without follicle stimulating hormone priming. Hum Reprod. 1999;14(7):1847-1851.
15. Shalom-Paz E, Holzer H, Son W, Levin I, Tan SL, Almog B. PCOS patients can benefit from in vitro maturation (IVM) of oocytes. Eur J Obstet Gynecol Reprod Biol. 2012;165(1):53-56.
16. Smith GD. In vitro maturation of oocytes. Curr Womens Health Rep. 2001;1(2):143-151.
17. Andersen CY, Leonardsen L, Ulloa-Aguirre A, Barrios-De-Tomasi J, Moore L, Byskov AG. FSH-induced resumption of meiosis in mouse oocytes: effect of different isoforms. Mol Hum Reprod. 1999;5(8): 726-731.
18. Zelinski-Wooten MB, Hutchison JS, Hess DL, Wolf DP, Stouffer RL. Follicle stimulating hormone alone supports follicle growth and oocyte development in gonadotrophin-releasing hormone antagonist-treated monkeys. Hum Reprod. 1995;10:1658-1666.
19. Eppig JJ. Gonadotropin stimulation of the expansion of cumulus oophorum isolated from mice: General conditions for expansion in vitro. J Exp Zool. 1979;208(1):111-120.
20. Strickland S, Beers W. Studies on the role of plasminogen activator in ovulation. In vitro response of granulosa cells to gonadotropins, cyclic nucleotides, and prostaglandins. J Biol Chem. 1976;251(18): 5694-5702.
21. Humaidan P, Bredkjaer HE, Bungum L, Bungum M, Grøndahl ML, Westergaard L, et al. GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: A prospective randomized study. Hum Reprod. 2005;20(5):1213–1220.
22. Humaidan P, Alsbjerg B. GnRHa trigger for final oocyte maturation: is HCG trigger history? Reprod Biomed Online. 2014;29(3):274–280.
23. Imoedemhe DA, Sigue AB, Pacpaco EL, Olazo AB. Stimulation of endogenous surge of luteinizing hormone with gonadotropin-releasing hormone analog after ovarian stimulation for in vitro fertilization. Fertil Steril 1991;55(2):328-332.
24. Oktay K, Turkcuoglu I, Rodriguez-Wallberg KA. GnRH agonist trigger for women with breast cancer undergoing fertility preservation by aromatase inhibitor/FSH stimulation. Reprod Biomed Online. 2010;20(6):783–788.
25. Reddy J, Turan V, Bedoschi G, Moy F, Oktay K. Triggering final oocyte maturation with gonadotropin-releasing hormone agonist (GnRHa) versus human chorionic gonadotropin (hCG) in breast cancer patients undergoing fertility preservation: an extended experience. J Assis Reprod Genetics. 2014;31:927-932.
26. Shavit T, Ellenbogen A, Michaeli M, Kartchovsky E, Ruzov O, Paz ES. In-vitro maturation of oocytes vs in-vitro fertilization with a gonadotropin-releasing hormone antagonist for women with polycystic ovarian syndrome: can superiority be defined?. Eur J Obstet Gynecol Reprod Biol. 2014;179:46-50.
27. Hachem ELH, Sonigo C, Benard J, Presse M, Sifer C, Sermondade N, et al. Comparison of GnRH agonist and hCG for priming in vitro maturation cycles in cancer patients undergoing urgent fertility preservation. PLoS One. 2018;13(12):e0208576.
28. Dahan MH , Zhang L, Chen HY , Tan SL. Early short stimulation modified natural cycle IVF with GnRH agonist trigger and in vitro maturation in a woman with polycystic ovary syndrome: A case report. J Obstet Gynaecol Can. 2016;38(5):465-469.
29. Galway AB, Lapolt PS, Tsafriri A, Dargan CM, Boime I, Hsueh AJW. Recombinant follicle-stimulating hormone induces ovulation and tissue plasminogen activator expression in hypophysectomized rats. Endocrinol. 1990;127(6):3023–3028.
30. Atef A, François P, Christian V, Marc-André S. The potential role of gap junction communication between cumulus cells and bovine oocytes during in vitro maturation. Mol Reprod Dev. 2005;71(3):358–367.
31. Christenson LK, Stouffer RL. Follicle-stimulating hormone and luteinizing hormone/chorionic gonadotropin stimulation of vascular endothelial growth factor production by macaque granulosa cells from pre- and periovulatory follicles. J Clin Endocrinol Metab. 1997;82(7): 2135–2142.
32. Reich R, Miskin R, Tsafriri A. Follicular plasminogen activator: involvement in ovulation. Endocrinol. 1985;116(2):516–521.
33. Yu B, Ruman J, Christman G. The role of peripheral gonadotropin-releasing hormone receptors in female reproduction. Fertil Steril. 2011;95(2):465-473.
34. Casper RF. Basic understanding of gonadotropin-releasing hormone-agonist triggering. Fertil Steril. 2015;103(4):867-869.
35. Ellenbogen A, Atamny R, Fainaru O, Meidan E, Rotfarb N, Michaeli M. In vitro maturation of oocytes: A novel method of treatment of patients with polycystic ovarian syndrome undergoing in vitro fertilization. Harefuah. 2011;150:833-836.