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Maturation Arrest of Oocytes – Case Reports

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Abstract

We report three case of maturation arrest of oocyte two of which were treated with oocyte donation. A total failure of human oocytes to complete meiosis is rarely observed during assisted conception cycles. Maturation arrest of oocytes may occur at various stages of the cell cycle from germinal vesicle (GV) stage to metaphase one and two (MI and MII).

Two cases that presented to us had unexplained infertility, but the third case had rheumatoid arthritis, which may result in an immune problem. In all the three couples, maturation arrest of the oocytes was noted repeatedly in all cycles of assisted reproductive technology (ART). In the first case the oocytes were arrested at both, the GV and metaphase I stage, in the second and third case arrest was noted in MI.

Failure to resume meiosis in vivo may arise at one of the following three levels:

✓ absent or incomplete luteinizing hormone(LH) effect

✓ derangements in the signaling mechanism from the surrounding cumulus cells

✓ intrinsic oocyte factors Currently no therapeutic approach would help to overcome the blocks in oocyte maturation and sustain successful in-vitro fertilization (IVF)

Oocyte maturation arrest may be the cause of infertility in some couples previously classified as having unexplained infertility. The recognition of oocyte maturation arrest as a specific factor for infertility tells, how important the oocyte factor is.

All the three patients were advised oocyte donation as the literature quotes very poor pregnancy rate with own oocyte. One patient conceived in the second cycle, whereas the other patient failed to conceive, despite transfer of good quality embryos.

Keywords: Agglutination; Rheumatoid arthritis; Oogenesis; Infertility

Maturation Arrest of Oocytes - Case Reports

Introduction

Maturation arrest of human oocytes may occur at various stages of the cell cycle. A total failure of human oocytes to complete meiosis is rarely observed during assisted conception cycles. Oocyte maturation arrest may be the cause of infertility in some couples previously classified as having unexplained infertility. The recognition of oocyte maturation arrest as a specific medical condition may contribute to the characterization of the yet poorly defined entity currently known as 'oocyte factor'. The cellular and genetic mechanisms causing oocyte maturation arrest should be the subject of further investigation.

Maturation arrests in human oocytes can oocur at (Figure 1)

1. Germinal vesicle (GV) stage when oocytes are awaiting the gonadotrophin signal or the release from an inhibitory follicular environment

2. At metaphase one (MI) and were unable to complete meiosis up to metaphase two (MII)

3. Oocytes did not respond properly to fertilizing sperm [1-5]

The figure below tells about expected happenings and potential points of arrest of the oocyte maturation.

Here we describe two such cases, where the maturation arrest of oocytes occurred at MI stage.

Case Report 1: MG a 30 year old patient married since 5 years presented with primary infertility. She had regular menstrual cycle and had contraception for 1½ years. HSG done in May 2003 was normal. Base line USG, serum prolactin and thyroid profile were normal. Semen analysis was normal except for agglutination of sperms. She had undergone ovulation induction (OI) for intrauterine insemination (IUI) with Clomiphene citrate (CC) for 3 cycles followed by with CC and human menopausal gonadotropin (hMG) for one cycle. Since no conception after four cycles of IUI, it was decided by the treating gynecologist to perform a hysterolaproscopy in March 2005. Hysteroscopy was normal but cervical stenosis was noted. At laparoscopy, endometriotic deposits were seen in the pouch of Douglas (POD), uterosacral ligaments and surface of both ovaries, which was fulgurated. Both tubes were normal and patent.

Gonadotrophin releasing hormone (GnRh) agonist depot was given for three months after laparoscopy. Following which she underwent five more cycles of IUI with OI with gonadotropins.

When she visuted our centre on examination BP was 120/70 mm of Hg, there was no hirsuitism, galactorrhoea and obesity observed. Per abdominal and per speculum examination was normal.

On per vaginal examination cervix was directed downwards and

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backwards. Uterus was anteverted, normal size, smooth firm mobile and both fornices were clear.

On baseline ultrasonography (USG): Uterus 67 × 29 × 43 mm in size with endometrial thickness of 6.9 mm (Figure 2). Ovarian volume was 6.8 cu mm on right side with 4-5 follicles and 7.4 cu mm on left side with 6 small follicles.

Day 2 hormonal profile was as follows:

Follicular stimulating hormone (FSH) 5.0 mIU/ml

Luteinizing hormone (LH) 5.3 mIU/ml

Dehydroepiandrosterone sulfate (DHEAS) - 1990 ng/ml

Androstenedione (ASD) - 1.45 ng/ml

Prolactin - 36 ng/ml

Thyroid stimulating hormone (TSH) - 2.8 uIU/ml

Semen analysis

Total count - 118 mill/ml

Total Motility 67%

Rapid linear (RL) 43%; Slow linear (SL) 15%; Non progressive (NP) 9% and Immotile (IM) 33%

Viability 70% with hypo osmotic swelling test (HOS) of 61%

Normal morphological forms were 17% with teratozoospermic index (TZI) of 1.40

Head to head agglutination was seen in few fields. Anti-sperm Antibody test was positive in both serum and semen.

She was advised to undergo IVF as she had history of several failed IUI cycles, but wanted to try few more cycles of IUI.

Hence three more cycles IUI were done

1. 1 with CC.
2. 1 with Letroz
3. 1 with GnRha+FSH

Post wash count was between 28 mill/ml, 18 mill/ml and 40 mill/ml with 100% rapid linear sperms in the three consecutive cycles. As there was no conception she agreed for in vitro fertilization (IVF)

She was down regulated with Leupride 250 mcg twice daily till day 2 and then the GnRH agonist was continued once a day. On day 2, her FSH was 2.6 mIU/ml; LH was 1.2 mIU/ml; Estradiol (E2) was 22 pg/ml; Progesterone (P4) 1.8ng/ml and Prolactin 2.75ng/ml.

On day 2 once down regulation was confirmed controlled ovarian stimulation (COS) was started with FSH 150 IU and hMG 75 IU. On day 4 her E2 was 190 pg/ml. On day eleven 5000 IU human chorionic gonadotrophin (hCG) given intramuscular (IM), when 5 follicles were

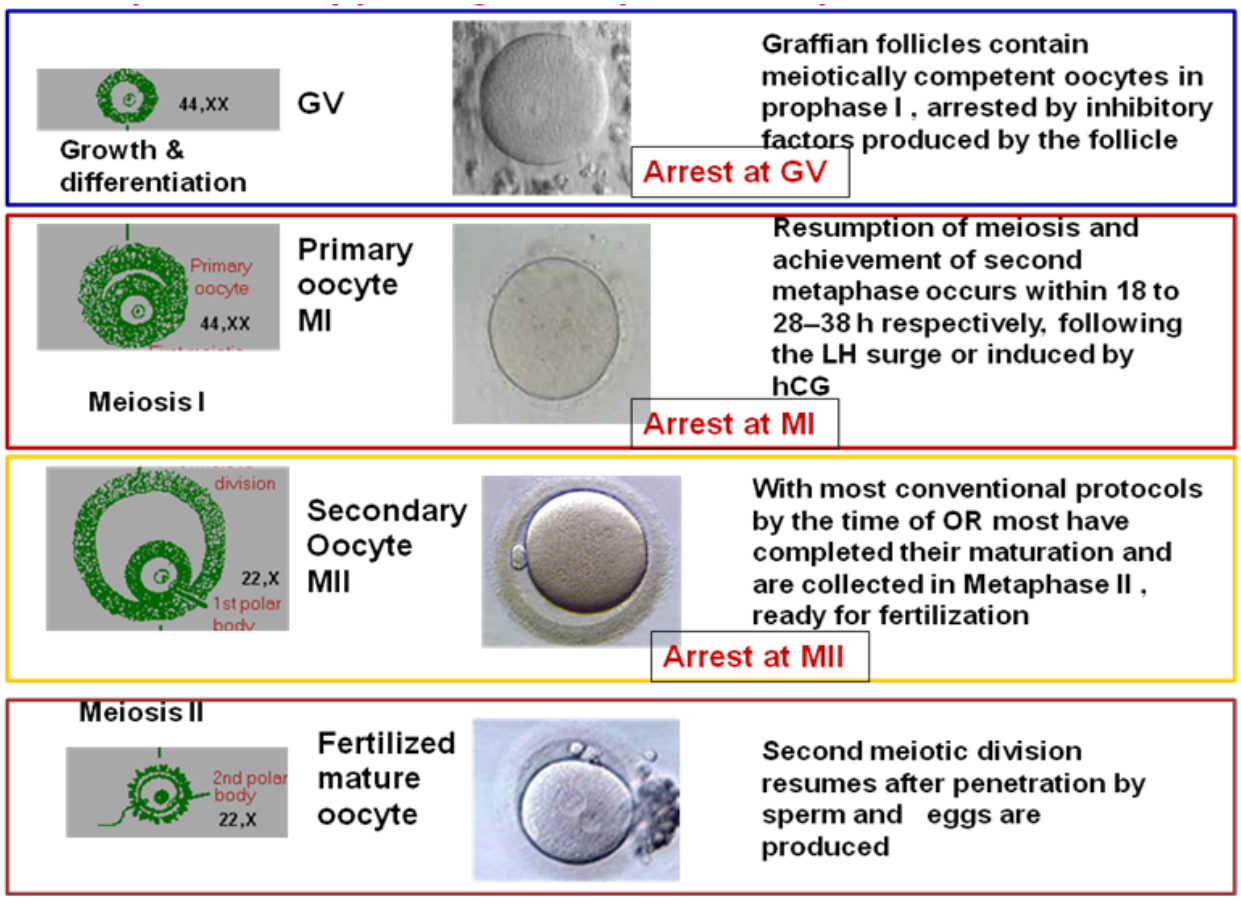

Figure 1: Stages of maturation arrest of oocytes in humans.

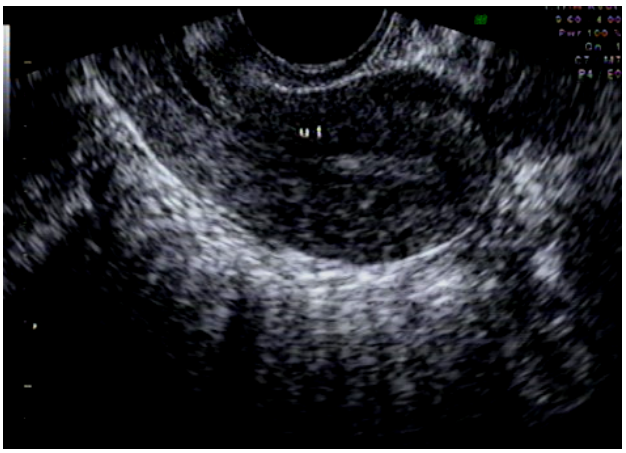

Figure 2: Transvaginal ultrasound of uterus.

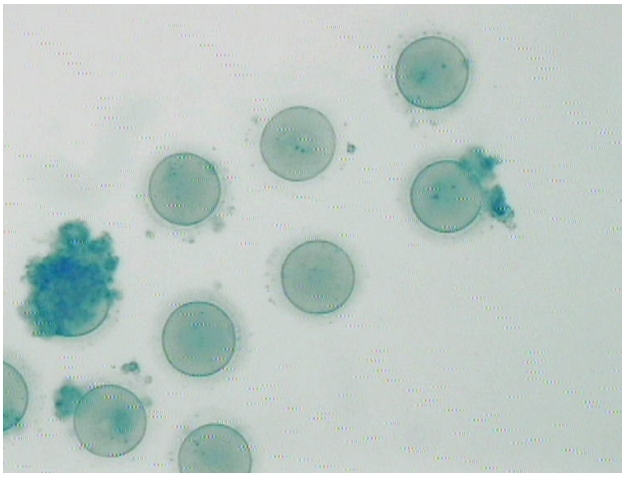

Figure 3: Immature oocytes.

between 16 –18 mm and 8 follicles 15–16 mm. On day of hCG the E2 was 3000 pg/ml, P4 was 0.9 ng/ml and the endometrial thickness was 12.5 mm.

Oocyte retrieval (OR) done 35 hours later, 13 oocyte cumulus complexes (OCC) obtained, which looked normal. Post wash count was 40 mill/ml 100% RL. IVF was done. After 18 hours when checked for fertilization, there was no fertilization as all eggs were immature (1 GV and 12 MI) (Figure 3).

Oocytes were further cultured in in vitro oocyte maturation (IVM) media for further 24 hours but failed to become MII (Figure 4).

Pure Progesterone was discontinued and Karyotype of both partners was sent for, which was normal.

Possible options which may help in over coming the block in oocyte maturation are

1. Administration of hCG at a follicular diameter>20 mm
2. Extending hCG to retrieval interval
3. Increasing the dose of hCG
4. Extended in vitro culture
5. Oocyte donation

We decided to do the next cycle with hCG being given at follicular diameter of 20–22 mm with higher dose of hCG.

Hence down regulation was started with Leupride 250 mcg SC from day 21. On day 2 FSH was 3.94 mIU/ml, LH 1.36 mIU/ml, E2 27 pg/ml and Progesterone was1.23 ng/ml. COS with Rec. FSH 225 IU SC. Estradiol on day 4 was 564.29 pg/ml thus dose was reduced to 150 IU. Estradiol level was 2800 pg/ml when there were 6 follicles 18 mm and 7 follicles 15–16 mm in diameter recombinant human chorionic gonadotrophin (Rec. hCG) 500 mg was given subcutaneously (SC) when maximum diameter of 7 follicles was between 20-22 mm. Estradiol was 4350 pg/ml, P4 1.3 ng/ml and endometrial thickness was 12.7 mm on the day of hCG. Oocyte retrival was done 36 hours later.

This time we decided to do intra-cytoplasmic sperm injection (ICSI), so that the maturity of the oocytes can be evaluated. 13 oocyte cumulus complexes were obtained. To our dismay this time also all oocytes were MI. Despite culturing them in in vitro maturation media, none of them matured even after 24 hours. All were still MI, 24 hours after in-vitro culture. E2 levels nor the endometrial thickness are predictable of maturity of oocytes (Figure 5).

Currently no therapeutic approach helps to overcome the blocks in oocyte maturation and sustain successful IVF. Currently, oocyte donation seems to be the most viable option.

Oocyte donation cycle

She under went one cycle of fresh and one of frozen embryo transfer (FET) done with donor oocytes. In fresh cycle ICSI was done on 7 MII oocytes and all 7 fertilized.

Day 3 – one 10 cell Grade A; Three 8 cell (1 - Grade A; 2 - Grade B); one 7 cell Grade B; one 6 cell Grade C with 40% fragmentation and one 4 cell Grade D with 70% fragmentation. Five were cultured for blastocyst.

2 Grade 4AA blastocyst were transferred on day 5 at 117 hours and two blastocyst Grade 3 AA and Grade 2 were frozen (Figure 6).

There was a biochemical pregnancy with beta hCG of 139 and 233 mIU/ml.

FET done in a natural cycle with progesterone support and two blastocysts (Grade 4 AA and Grade 2) transferred (Figure 7).

Successful pregnancy was confirmed by rising Beta hCG levels from 327 mIU/ml to 3161 mIU/ml, 10600 mIU/ml, 1,19,050 mIU/ML and Presence of Gestational sac at 5 weeks 2 days with fetal pole and heart beat at 6 weeks (Figure 8).

She delivered at 38 weeks a male child weighing 2.9 kg with normal Apgar scores. The child has normal developmental milestones and is seven years old.

Case 2: 31 year old AJ presented to us with inability to conceive for last four years. Her menstrual cycles were regular and normal with the coital frequency of three per week. She did not have any significant past medical history and had undergone hysteroscopy before coming to us, which was normal. She had taken Clomiphene citrate for three cycles with timed intercourse, one cycle Letrozole with timed intercourse, two cycles of IUI with gonadotropin and one cycle of IVF the details of which were not available with the patient.

On examination she was not obese and did not have any features of

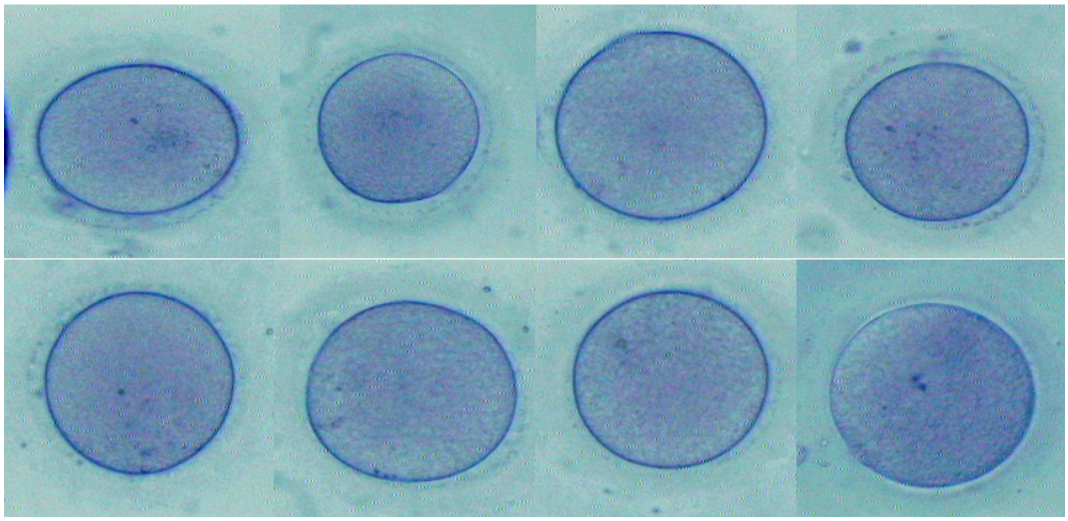

Figure 4: Immature even after IVM.

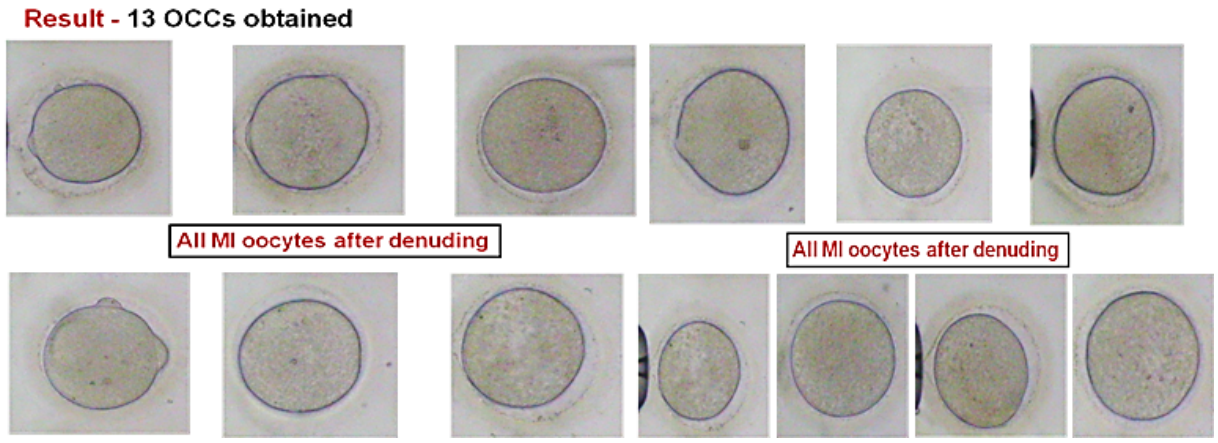

Figure 5: Immature oocytes at the second cycle of IVF.

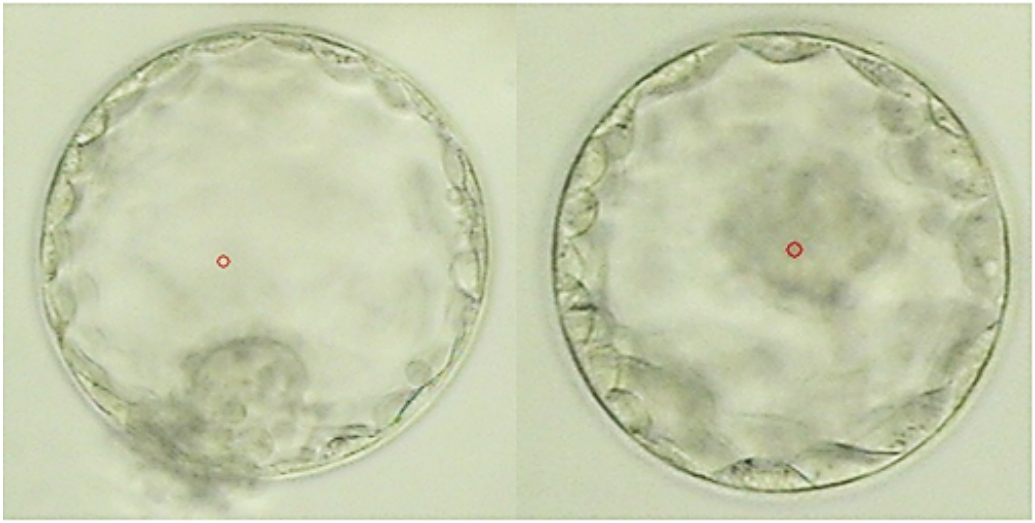

Figure 6: Blastocysts transferred at Fresh IVF cycle.

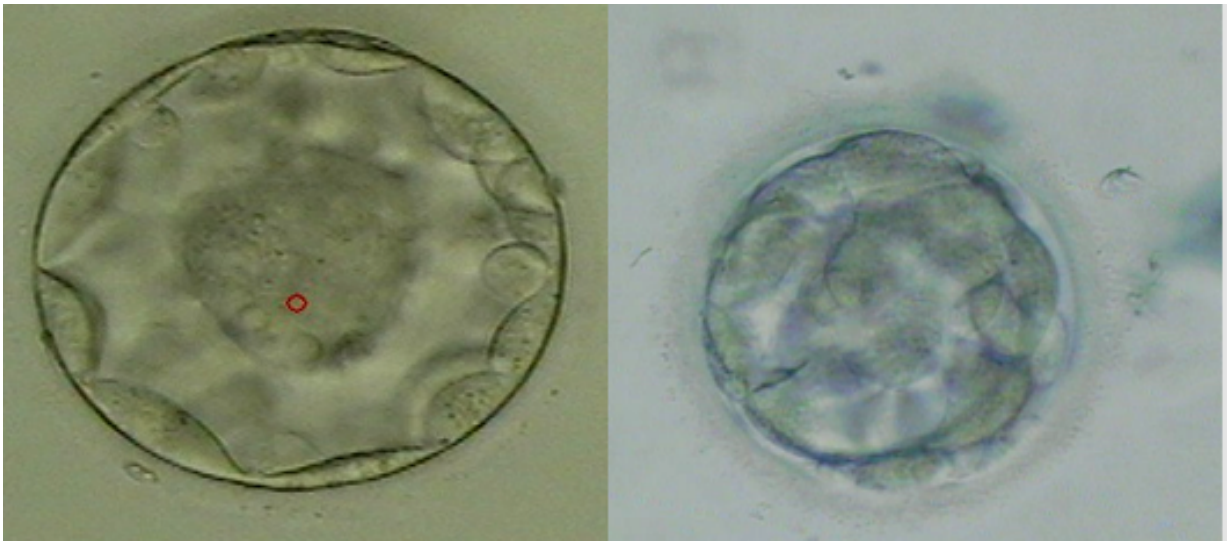

Figure 7: Blastocysts transferred at FET.

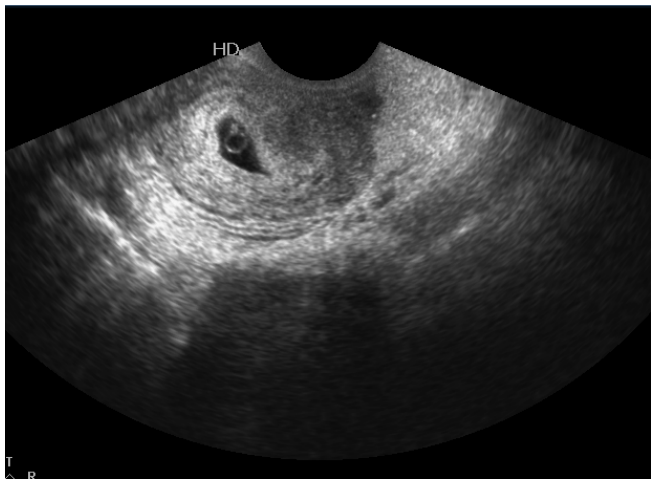

Figure 8: USG picture of live pregnancy at 6 weeks.

androgen excess or galactorrhoea. There was no abnormality detected on per abdominal and per speculum examination. On per vaginal examination cervix was directed downwards and backwards; Uterus anterverted normal size, firm, smooth and mobile with both fornices being clear.

At baseline USG the uterus was 77.7 × 31.4 × 44 mm with endometrial thickness of 6.4 mm. Right ovary measured 29.6/26.3/26 mm with a volume of 10.5 $cm^3$and 7 small follicles. Left ovary was 20.6/21.2/20 mm with a volume 4.57 cm^3 and 3 small follicle.

Day two hormonal profile was as follows

FSH 7.03 mIU/ml LH 3.26 mIU/ml;

Anti mullerian hormone (AMH) 0.72 ng/ml

DHEAS - 2950 ng/ml ASD - 2.9 ng/ml

Prolactin - 16.97 ng/ml TSH - 1.8 uIU/ml Vitamin D3 - 9.1 ng/ml

Semen analysis showed a normal total count of 80 mill/ml. The motility was decrease with a total being 42%. Of these 30% had rapid linear motility, 9% slow linear, 3% non-progressive and 58% were immotile. The viability 65% with HOS of 60%. Morphologically normal forms were 14% with a TZI of 1.62. 24 and 48 hours sperm survival was 85 and 80% respectively.

One cycle of IUI was done with gonadotropins 75 IU in a GnRH agonist short protocol. She had 3 dominant follicles on day 11 of stimulation when Rec. hCG 250 mcg was given SC and IUI was done 36 hours later. The post was count was 100 million/ml with 100% rapid linear motility. As she did not conceive IVF was planned in the next cycle in an antagonist cycle. Her day two hormonal reports were as follows:

FSH- 4.9 mIU/ml; LH - 3.2 mIU/ml; E2 - 42 pg/ml and Progesterone of 10.6 ng/m.

COS was started with Rec FSH 150 IU. On Day 4 of COS, E2 value was 463 pg/ml despite the presence of only 7 small follicles between 6-10 mm. GnRH antagonist 0.25 mg was started in view of a high E2 level on day four. Rec. hCG 250 mcg was given SC on day 11 of menstrual cycle and day 9 of COS when there were 6 dominant follicles 18–19 mm in diameter. On day of hCG the estradiol and progesterone values were 2218 pg/ml and 0.7 ng/ml respectively. The endometrial thickness was 12.6 mm. OR was done 35 hours later, 6 OCC obtained which looked normal. Post wash count was>100 mill/ml 100% RL, so IVF was done. After 18 hours when checked for fertilization, there was no fertilization as all oocytes were MI (Figure 9). We kept these oocytes in IVM medium for further 24 hours, but none of them matured.

Karyotyping was done, which showed 46 XX, 21p+. She was sent to a genetic counseling and was told that this anomaly has no effect on the conception.

In view of previous maturation arrest we decided to use a short GnRH agonist protocol, and Recombinant FSH in the dose of 200 IU was started on day three of menstrual cycle. E2 on day 4 of COS was 467 pg/ml. Injection hCG 10,000 IU was given on day 12 of menstrual cycle and day 10 of COS, when 6 follicles were 20 to 21 mm and 4 follicles were 17–19 mm in diameter. Her E2 on day of hCG was 4708 pg/ml and P4 was 1 ng/ml. OR was done 36 hours later, 9 oocytes

obtained. The cumulus complexes were denuded to know the status of the oocytes and to our dismay all were MI. We did keep them in oocyte maturation media but none of them progressed to MII.

One common thing in both her cycles was that the estradiol level on day 4 of COS and on the day of hCG was high and did not correspond to the number of follicles in both cycles.

She has been counseled for oocyte donation, the first cycle was done in a hormone replacement cycle (HRT), which did not result in a pregnancy despite transfer of 2 good quality embryos (4AA and 3AA) (Figure 10).

Case 3: DA a 30 years old patient married for 6 years and trying to conceive for 4 years was referred for further management in 2014. Her menarche was at 15 years and had regular menstrual cycles. She had used oral contraceptive pill for 2 years. She was diagnosed with rheumatoid arthritis in 2010 and had taken sulfasalazine and hydroxychloroquine (HCQ) for 4 years. She was on some Ayurvedic medicine for 4 months. There was a family history of paternal aunt suffering from rheumatoid arthritis. She had undergone a hystero-laparoscopy with ovarian drilling in March 2011. After that she had undergone six cycles of IUI with CC and two cycles of ICSI before being referred to us. The post-wash count was between 22 to 31 million with 100% rapid linear motility. Both the ICSI cycles were done an antagonist cycle and the Rec. FSH 150 IU and hMG 75 IU was given for stimulation. In the first cycle the patient had moderate ovarian hyper stimulation syndrome (OHSS) but all the oocytes retrieved were immature. Patient did not have details of the number of oocytes aspirated and whether the oocytes were arrested in GV or MI. There was also no information on the second cycle of ICSI.

On examination, there were no signs of androgen excess and galactorrhea. There was no abnormality detected on per abdominal and per speculum examination. On per vaginal examination cervix was directed downwards and backwards; Uterus was anteverted normal size, firm, smooth and mobile with both fornices being clear. At baseline USG the uterus was 66.5 × 28.1 × 38.5 mm with endometrial thickness of 7.7 mm. Right ovary measured 21.9/20.3/18.8 mm with a volume of 4.37 $cm^3$and 5 small follicles. Left ovary was 20.3/19.1/21.3 mm with a volume 4.12 cm^3 and 2 small follicle.

Day two hormonal profile was as follows

FSH 3.65 mIU/ml LH 1.48 mIU/ml; AMH 2.3 ng/ml

Prolactin - 17.1 ng/ml TSH - 1.93 uIU/ml Vitamin D3 – 78 ng/ml

Antiphospolipid (APLA) screening done was negative but the RA factor was positive

Karyotype was normal with satellite on Chromosome 15 (46xx 15ps (+)), genetic counselling done for the same.

Semen analysis showed a normal total count of 192 mill/ml. The motility was decrease with a total being 49%. Of these 2% had rapid linear motility, 38% slow linear, 9% non-progressive and 33% were immotile. The viability 70% with HOS of 70%. Morphologically normal forms were 19% with a TZI of 1.4.

As the patient had undergone six cycles of IUI and 2 cycles of ICSI, which had failed, she was counseled for ART.

On Day 2 of menstrual cycle, FSH - 13.5 mIU/ml , LH - 6.99 mIU/ml, E2- 52 pg/ml, P4 - 0.3 ng/ml. COS was started with Rec FSH 150 IU and hMG 150 IU in a short agonist protocol. On day 4 of COS her estradiol value was 245 pg/ml and so the same dose was continued. On day 13 of COS 250 mcg of Rec hCG was given subcutaneously, when there were 5 dominant follicles between 21 -22 mm (larger diameter as history of immature oocytes at OR). On the day of hCG the estradiol value was 2371 pg/ml and that of progesterone was 1.1 ng/ml. OR was done 36 hours later. Six oocytes were obtained. After denudation the oocytes appeared extremely shriveled with thick zona pellucida (ZP). These oocytes (Figure 11), which were either GV (2) or MI (4) were cultured for 48hrs in IVM media, despite which none of them matured.

In view of obtaining immature oocytes in repeated cycles, patient was counseled for oocyte donation, for which she is not willing.

The table below gives the basic parameters of all the three patients (Table 1).

Discussion

Follicular somatic cells within the cumulus–oocyte complex suppress the expression of meiotic competence in oocytes *in vivo*, and also mediate the stimulation of meiotic maturation and its extent. A correct nuclear and cytoplasmic maturation of the oocytes is essential for normal fertilization and male nucleus decondensation to occur, and thus permit subsequent embryo development. The potential of the oocyte to progress through meiosis is acquired only after certain structural and biochemical changes in the various compartments of the nucleus and cytoplasm [6].

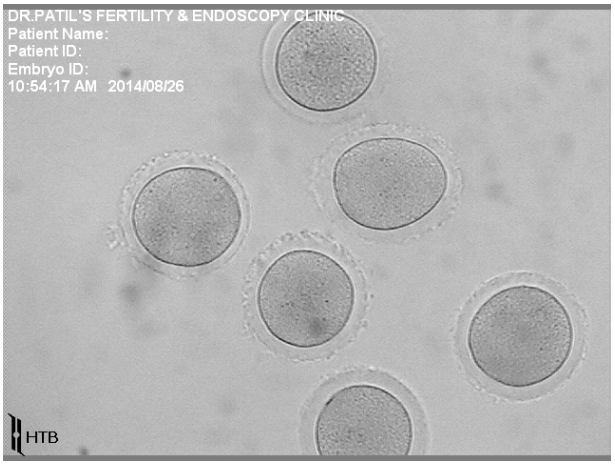

Figure 9: Immature oocytes at 18 hours after insemination.

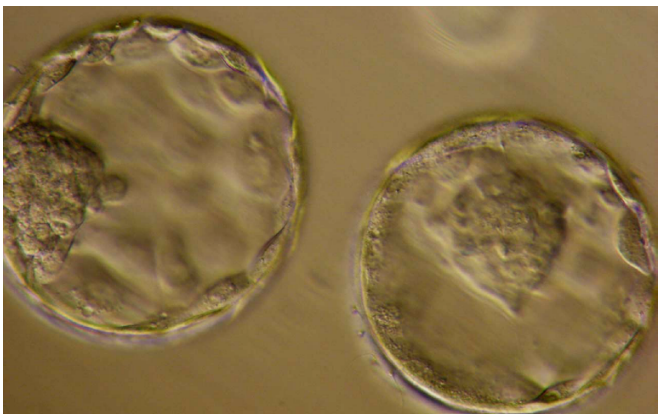

Figure 10: Embryos transferred.

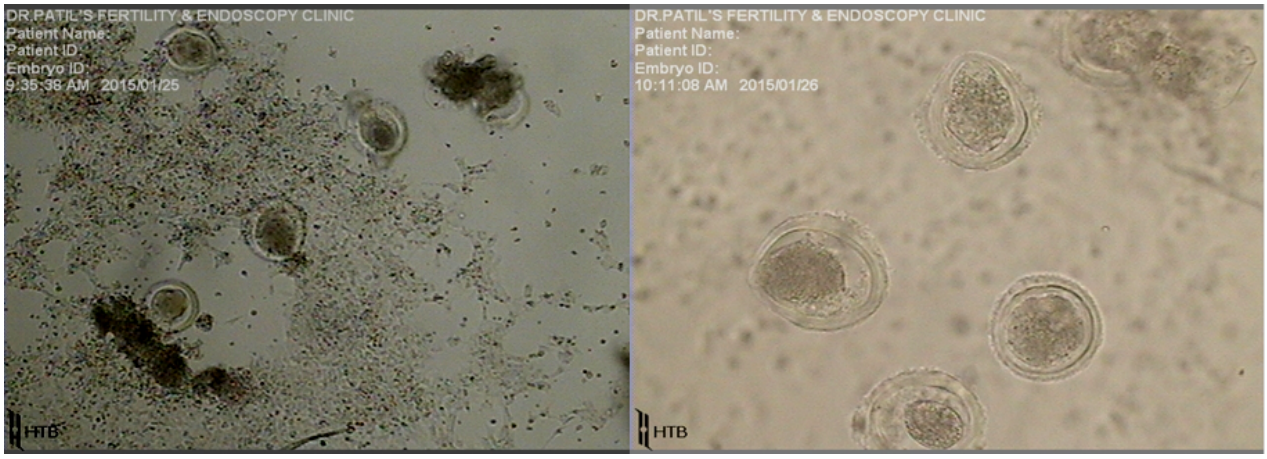

Figure 11: GV and MI, shriveled oocytes retrieved.

Parameter	Case 1	Case 2	Case 3
Age in years	30	31	30
Menstrual cycle	Regular	Regular	Regular
FSH (mIU/ml)	5	7.03	3.65
LH (mIU/ml)	5.3	3.26	1.48
AMH (ng/ml)	-	0.72	2.3
Semen Analysis	Normal	Normal	Normal
Total count (mill/ml)	118	80	192
Progressive Motility	67	39	40
Morphology %	17	14	19
Viability %	70	65	70
TZI	1.4	1.62	1.4
E2 on day of hCG (pg/ml)	3000 4350	2218 4708	2371
P4 on day of hCG (ng/ml)	0.9 1.3	0.7 1	1.1
Number of oocytes	13 13	6 9	6
GV	1 0	0 0	2
MI	12 13	6 9	4
MII	0 0	0 0	0
Number matured in vitro	0 0	0 0	0

Table 1: Demographic details of the 3 cases.

Changes in the cytoplasm include modification of Golgi complex, accumulation of ribosomes and increase in number and change in morphology of mitochondria [7]. Changes in the nucleus are transition from a diffuse, reticulated configuration to dense uniform body and reflect a period of intensive RNA synthesis [7].

Oocyte developmental competence is gradually acquired during the long-lasting period of oogenesis. Final stage for optimal development, prior to ovulation, requires synchronization between nuclear and cytoplasmic maturation.8 Use of hormones for COS results in follicular heterogeneity and, consequently, in oocytes with diverse stages of nuclear and cytoplasmic development [8]. Hence, different chromatin configurations within the population of fully grown GV oocytes were reported [9,10]. Growing oocytes are unable to respond to maturation signals both in vivo and in vitro and remain arrested in diplotene stage or if more advanced progress only to metaphase I. Only fully-grown oocytes respond to gonadotrophic signals and mature to metaphase II oocytes in the pre-ovulatory follicles or in culture media [8-10].

Failure to resume meiosis *in vivo* may be due to_absent or incomplete LH effect, derangements in the signaling mechanism from the surrounding cumulus cells and intrinsic oocyte factors.

Intrinsic oocyte abnormality may result in unresponsiveness of growing oocytes unable to maturation signals both in vivo and in vitro and remain arrested in the diplotene stage or, if more advanced, progress only to metaphase I. Deranged regulation of MPF activity or abnormal spindle formation are responsible for the meiotic arrest at metaphase I. Factors involving abnormal cell cycle control, spindle and/or cytoskeletal function may also contribute to failures in progression of oocyte from metaphase I (MI) to metaphase II (MII). This could be due to failure of formation or maintenance of the bipolar spindles. There could also be some genetic disorder in which human oocytes are

incapable of completing meiosis [5,11,12].

The process of maturation of the oocyte is under control of maturation promoting factor (MPF), which is an M-phase specific kinase resident in oocytes that must be activated for meiotic resumption. When cyclin (regulatory subunit of MPF) degradation is inhibited, MPF remains active and oocytes reach MII. Resumption of meiosis after fertilization is associated with loss of kinase activity that could be regulated by intracellular calcium oscillation and loss of MPF activity. This results in division of metaphase arrested chromosomes resulting in extrusion of second polar body, leaving behind a haploid set of chromosomes [13]. In immature oocytes, MPF is present in an inactive phosphorylated form as a complex of Cdk 1 and cyclin B. This phosphorylation is controlled by Myt 1 kinase. The dephosphorylation of MPF is induced by Cdc25 phosphatase (probably by Cdc25B). Activity of MPF reaches its peak in MI oocyte and then decreases during the anaphase to telophase transition. Thereafter, high levels of MPF are again restored and oocytes are kept at this stage under the infuence of a cytostatic factor (CSF) [14]. Once MPF is activated, this kinase phosphorylates an array of protein targets that lead to significant remodeling of nuclear and cytoplasmic compartments during oocyte maturation. It has been observed that MPF is fully degraded when oocytes are fertilized or parthenogenetically activated [15,16] physiological control mechanisms intrinsic to oocyte and involving MPF expression and activation must be modulated precisely during follicle growth and ovulation to coordinate these events with meiotic maturation.

The process of maturation, however, is much more complex and not yet fully understood [17]. Follicle attain the appropriate size at the time of aspiration whilst the oocyte may still slightly developmentally behind and the chromatin configurations differ in immature human oocytes collected from large antral oocytes. This means that follicle size is not an indicator and does not correspond to oocyte matuartion.

Only those oocytes in which the nucleolus is surrounded with a ring of condensed chromatin mature better and are more developmentally competent after fertilization [9,10,18]. Inability of oocytes to mature was observed repeatedly, in some rare heritable molecular defects that are responsible for the inability of these oocytes to initiate the activation of MPF [4].

Metaphase I arrest both in vivo and in vitro may be due to inability of oocytes to attain the full competence to mature. It result from the absence of meiotic recombination, which under normal conditions, occurs in pachytene stage and inability of oocytes to produce the key cell cycle regulating factors - responsible for a correct spindle function, play a crucial role in the transition either from MI or MII [19-21]. The progression from MI to anaphase require proteolysis of cyclin molecule along with microfilaments [22], which also play an important role because they can influence the migration of spindle to the oocyte periphery. Deranged regulation of MPF activity or abnormal spindle formation may also be responsible for meiotic arrest at MI.

Metaphase II arrest and abnormal situations after fertilization may also be observed. Here oocytes were collected in MII stage and after ICSI two pronuclei were detected in them but there was no extrusion of the second polar body. This indicates some MII spindle abnormalities as there is a very high probability that the separation of individual chromatids occurred as a consequence of oocyte activation but was not extruded. There could also be failure of oocyte activation, which prevents the formation of both pronuclei and the sperm head chromatin does not undergo the decondensation [6]. In aged oocytes the metaphase spindle is not located at the periphery and moves to the oocyte centre.

One must also remember that oocytes matured in vitro do not have the same developmental potential as in vivo matured oocytes as culture conditions adequately support nuclear maturation [23-26], but frequently fail to sustain optimal cytoplasmic maturation [10]. This then accounts for a sub optimal outcome after ART.

Possible approaches which may help in over comimg the block in oocyte maturation are administration of hCG at a follicular diameter>20 mm, extending hCG to oocyte retrieval interval to more than 36 hours, increasing the dose of hCG and extended in vitro culture. In most cases these therapeutic modalities are without success and today oocyte donation is the most viable option. Possible approaches which may solve at least some of the problems in future are cytoplasmic transfer, germinal vesicle replacement, metaphase spindle transfer and IVM of immature oocytes in culture media enriched with yet undetermined factors necessary for oocyte maturation. Before their use in clinical practice it is important that they be tested in animal models. One should also keep in mind that with these approaches there is a chance of issues like `heteroplasmy' and `epigenetic modifications that can occur [27-29].

Rudak et al. [30] as early as 1990 reported three cases of which one was arrested at germinal vesicle stage (GV) and the other two at MII. Then in 1995 Eichenlaub-Ritter et al. [12] reported one case with recurrent failure of polar body formation and premature chromosome condensation. Hartshorne et al. [31], Barblett [32] and Zhi Chen et al. [33] reported one case each of oocytes arrested at GV and Harrison et al. [34] in 2000 reported two cases, where the oocyte was arrested in MII. Largest number of cases published was by David Levran et al. [4], who reported seven cases in 2002 of which four were arrested at MI, three MII and there was one case of derangement in meiotic and fertilization process. Combelles et al. [10] published two cases of maturation arrest, one had defects in spindle and chromatin arrangement whereas the other one had possible defect in the cell cycle control. Schmiady and Neitzel [5] reported two sisters born from consanguineous parents had unsuccessful IVF treatment due to maturation arrest of the oocytes. Their oocytes showed neither pronucleus or first or second polar body. Most of these patients were treated successfully with oocyte donation. Their two brothers also failed to have children, though the karyotypes of all four were normal. This probably may ne due to inhetitance of an autosomal recessive trait.

Conclusion

In our cases intrinsic oocyte abnormality seems to be the cause for maturation arrest as insufficient LH effect is ruled out as the arrest was at metaphase I and was repetitive. Moreover presence of normal ovarian steroidogenesis (normal estradiol and progesterone levels) before and after hCG injection rule out dysfunction of granulosa and theca cells. Ease of oocyte aspiration represents a normal LH effect. Two cases had satellite on Chromosome, in one case on chromosome 15 and in the other on chromosome 21.

Presence of mature cumulus cells with normal appearance that surrounded the oocytes which were normal in size as expected for mature oocyte and the ease with which oocytes were denuded rules our derangements in the signaling mechanism from the surrounding cumulus cells. Achievement of normal pregnancy with the use of donor oocyte suggests that the underlying abnormality was in the oocyte.

Thus, maturation arrest is not a sporadic event of abnormal

response to ovarian stimulation or poor culture conditions. It was observed repeatedly and probably arose from intrinsic oocyte abnormality; therefore oocyte donation was the only viable option in these patients with maturation arrest of oocytes.

As it is difficult to identify maturation arrest of oocytes unless and ART procedure is done, such women are subjected to extremes of financial, emotional and physical burden due to repeated failures of infertility treatment.

More information on the physiology and pathophysiology of oocyte maturation with knowledge of cellular and genetic mechanism causing oocyte maturation arrest is necessary before the exact nature of defects interfering with meiotic competence can be determined and effective therapy can be suggested. Based on a candidate gene approach, the genetic or microarray analysis of patients will help to identify genes and pathways involved in complete failures [35-39]. One can infer from these cases that "oocyte factor" probably may explain the reason for "unexplained infertility". So patients with unexplained infertility should proceed with IVF instead of ovulation induction with timed intercourse or intrauterine insemination.

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