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## Original Research Article

# Study of patients with diminished ovarian reserve and our approach to their treatment: retrospective analytic study

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### ABSTRACT

**Background:** Diminished ovarian reserve (DOR) is a perplexing entity. For the physician, optimisation of all aspects of management is needed to fruitfully utilise the available pool of gametes and time.

**Methods:** In this multicentric retrospective analytic study, we re-evaluated all cases of DOR. All aspects of management were critically assessed.

**Results:** We saw that idiopathic DOR was the most common etiology. AMH titres are reflection of oocyte yield. Stop agonist-antagonist and micro dose flare gives a higher oocyte yield. More number of good quality blastocyst was available in antagonist cycles. Growth hormone as adjuvant is helpful in DOR cases.

**Conclusions:** DOR needs special care and urgency in treatment. Appropriate selection of protocol and adjuvants to treatment gives adequate pregnancy rate.

**Keywords:** Diminished ovarian reserve, Stimulation protocol, Stop-antagonist protocol

### INTRODUCTION

Infertility is a global and evolving morbidity. The incidence is about 12-18%. With changes in life style and postponement of child birth the problem is further compounded. Diminished ovarian reserve is a multi-etiological issue. It affects about 10% of ladies seeking treatment.<sup>1</sup> Signs of DOR reflect reduced fertility. Treatment options are varied and need to be applied in a personalized manner. Age related decline in reproductive potential is due to oocyte and uterine aging compounded further by life style diseases. Uterine aging is reflected by poor endometrial growth. Obstetric outcome is guarded and needs close materno fetal surveillance.<sup>2</sup> Surgical insult may lead to DOR by inadvertently removing healthy ovarian tissues as well by causing vascular compromise. In all etiologies of DOR there is increased apoptosis. This exaggerated weakening of oocyte production and quality further becomes POF over variable time period.<sup>3</sup> This decline is dictated by age, genetics and environmental factors.

The objective of this study was to assess various interventions being done at our centers. Individual efficacy and factors affecting them were analysed.

### METHODS

This was a multicentric, retrospective, analytic study of all patients registered at two ART centers of armed forces, tertiary care hospital. All patients diagnosed to have DOR were included in the study. Namely with AFC below 5, poor ovarian response in previous cycles or with diminished AMH levels. Ladies with exposure to cytotoxic drugs and pelvic oncosurgery were excluded.

#### *Study location*

Multicentric study was carried out at (a) Basistha ART centre; (b) Navakriti ART centre for one year (01 January 2019 to 31 December 2019).

### Sample size

All patients registered for treatment at ART centre with Diminished Ovarian Reserve (DOR). Total of 329 couples were registered at these two centers.

### Stimulation protocol

Stimulation protocol was as follows- (i) Antagonist protocol: Patients were started on injection recombinant Follitropin Alpha (Merck Pharmaceuticals) 1050 IU/1.75 ml powder with solvent for stimulation from day 2 of menstrual cycle. Personalized stimulation protocols were started. Injection Ovurelix containing Citorelix 0.25 mg from Sun Pharmaceuticals was started on evidence of sufficient endogenous estrogen production. Namely ultrasound follow up showing follicular size reaching 12 mm or endometrial thickness more than 6 mm. On adequate stimulation with a cohort of at least 2 follicles of size 18 mm, ovulation trigger was given; (ii) Miniflare antagonist protocol: similar to antagonist protocol, but initial 3 doses of FSH were supplemented with injection Decapeptyl from Ferring Pharmaceutical containing injection Triptorelin 0.1 mg SC to provide flare of FSH and LH due to agonist effect; (iii) Agonist protocol: Pituitary down regulation was started in luteal phase of previous cycle. Oral contraceptive pills were started on day 5 of previous cycle and on D21 patient was called for a TVS. Patient started on Agonist for pituitary down regulation. Injection Decapeptyl 0.1 mg or leuprolide Acetate 1 mg (Luprofact 4 mg/4 ml from Bayer Zydus Pharma) and called on D2-3 for adequacy of down regulation. Patient was started on injection FSH based on personalized stimulation protocol; (iv) Stop agonist-antagonist protocol: Similar to agonist protocol, but agonist discontinued on starting injection FSH for stimulation; (v) ANDRO IVF protocol: Patient was started on subcutaneous testosterone alternate day on previous cycle day 2 with OC pills.<sup>4</sup> Aromatase inhibitor were started tablet Fempro from Cipla Pharmaceuticals containing Letrozole 2.5 mg daily, to prevent aromatization of androgen to estrogen. Stimulation was started on day 2 as for antagonist cycle. Ovulation trigger was provided. Injection HCG 10,000 IU or Recombinant HCG Ovitrelle 250 mcg/0.5 mcg SC from Merck Serono containing Choriogonadotropin or agonist trigger as injection Decapeptyl 0.2 mg sc for antagonist, stop and flare protocols or both were given. This was followed 36-40 hr later by OPU.

Ovum pickup was done by standard technique. Semen preparation was done using double density gradient method. Conventional insemination was done only if post wash specimen shows a sperm concentration of more than 20 million/ml with more than 50% grade 4 motility. Fertilization was assessed after denudation after 16-18 hours of insemination. Fertilization was assessed after 16-18 hours of insemination. Luteal support was provided with oral/vaginal Progesterone. Beta HCG was done on day 15. Values above 5 mIU/ml were used as positive for calculating implantation rate. Clinical pregnancy was defined as ultrasound detection of embryonic cardiac activity.

Chi square test was used for data analysis.

### Adjuvant to treatment

Adjuvant to treatment was- (a) DHEA in the dose of 25 mg three times a day for 10-12 weeks before starting treatment; (b) Injection growth hormone 4 IU SC with start of stimulation; and (c) Subcutaneous testosterone 10 mg daily or alternate day.

## RESULTS

### Distribution of DOR cases

In our study population, 12 % of cases had DOR. We could retrieve, fertilize and transfer embryos in 80% cases (36 of 45). Our implantation rate was 22%. Due to poor endometrium, three patients were deferred for later transfer (FET cycles). Most of the cases of DOR were idiopathic. 15 out of 45 cases were advanced reproductive age patients. Previous non-oncology surgery accounted for 15% of cases (Table 1-2).

**Table 1: Distribution of data.**

Legends	No. of patients
<b>Total patients</b>	367
<b>DOR</b>	45
<b>Cycle cancellation</b>	3
<b>Embryo transfers</b>	36
<b>Fertilization failure</b>	3
<b>Total pregnancies (pregnancy rate %)</b>	8 (22)
<b>No transfer (poor endometrium)</b>	3

**Table 2: Etiology DOR and outcome.**

Etiology	No. of patients (%)	MII/No. of oocytes retrieved (% mature oocyte)	Total Tr	Pregnancies (PR) (%)
<b>Idiopathic</b>	23 (51)	81/103 (78)	21	5 (23)
<b>Age related</b>	15 (33)	29/49 (59)	9	1 (11)
<b>Previous surgery</b>	7 (15)	57/69 (82)	6	2 (33)
<b>Total</b>	45	167/221 (75)	36	8 (22)

**AMH, Age and AFC distribution and their embryology aspects**

Highest yield of MII oocytes were in AMH above 1.1 ng/ml. 7 of 26 patients conceived in patients with AMH above 0.5 ng/ml. There was no day 5 transfer below AMH 0.5 ng/ml. Above AMH 0.5 ng/ml 7 of 26 transfers were good quality day 5 embryos. More than 80% (117 of 130) of oocytes below age 35 years were mature MII quality, whereas the percentage fell below 55% (50 of 91) above age 35 years. Maximum pregnancy rate was seen for ladies in the 25-30 years category, 33% (3 of 9 transfers) whereas

the rate was 20% (3 of 14 transfers) for ladies above age 35 years. 2 of 15 transfers (13%) were day 5 above 35 years with DOR as compared to 5 of 21 transfers (23%) below age 35. Mature oocyte yield was proportional to AFC in our study.

Below AFC 6, 58% (34 of 58 oocytes) were MII maturity as compared to 81% (133 of 163 oocytes) above AFC of 6. The pregnancy rate was 33% (6 of 18 transfers) for AFC above 6 as compared to 11% (2 of 18 transfers) for AFC above 6 (Table 3).

**Table 3: AMH, Age and AFC distribution and outcome.**

AMH	No. of patients	MI/No. of oocytes retrieved (% mature oocyte)	D2 Tr	D3 Tr	D5 Tr	Total Tr	Pregnancies (PR %)
Above 1.1	17	97/117 (82)	2	9	4	15	4 (26)
1.0 to 0.5	13	51/71 (71)	2	6	3	11	3 (27)
0.4 to 0.1	15	19/33 (57)	9	1		10	1 (10)
	45	167/221 (75)	13	16	7	36	8 (22)
<b>Age (years)</b>							
Below 25	5	39/41 (95)	0	3	2	5	1 (20)
25-30	10	49/56 (87)	0	6	3	9	3 (33)
30-35	8	29/33 (88)	4	3		7	1 (14)
35-40	9	18/29 (62)	3	4	2	9	2 (22)
40-45	7	21/39 (56)	5			5	1(20)
45 plus	6	11/23 (47)	1			1	Nil
	45	167/221 (75)	13	16	7	36	8
<b>AFC</b>							
1-3	11	9/21 (42)	5	1	0	6	0
4-6	13	25/37 (67)	4	4	4	12	2 (16)
7-9	14	52/70 (74)	4	6	1	11	3 (27)
10 and more	7	81/93 (87)	0	5	2	7	3 (42)
	45	167/221 (75)	13	16	7	36	8

**Stimulation protocol and outcomes**

15 of 45 patients (33%) underwent antagonist stimulation protocol. Modification of the antagonist protocol, Microdose flare and Stop protocol was followed in 23 patients (51%). Andro IVF protocol was followed in poor prognostic patients and those with previous cycle cancellation in our centers.

Duration of stimulation was lesser in antagonist-based protocol as compared to long protocol (11.6 days vs 13 days). Total gonadotropin dose was lesser in antagonist protocol as compared to agonist protocol. Average oocyte yield for antagonist-based protocol was 4.6 as compared to 3.2 for agonist protocol. Stop antagonist and antagonist protocol had maximum mature oocyte yield (84%). 7 of 32 transfers were day 5 for antagonist protocols. 49 of 65 MII oocytes (75%) fertilized in Stop agonist-antagonist protocol as compared to 9 of 23(39%) in agonist protocol.

Only 16% embryos (2 of 12 embryos) had more than 20% fragmentation in antagonist protocol.

In Stop protocol the proportion of embryos with more than 20% fragmentation was 23% (3 of 13 embryos). We could vitrify more oocytes in antagonist protocol cycles (Table 4 and 5).

**Adjuvant to treatment**

Growth hormone supplementation yielded a mature oocyte component of 67%. DHEA supplementation was commonly used in our clientele. Mature oocyte yield was higher in this group (151 of 183, 82%). 6 of 33 transfers were day 5, expanded blastocyst.

Topical steroid was used in 5 patients with prior cycle cancellation. In Recombinant LH group 7 of 10 patients underwent transfer on day 3 and beyond (Table 6).

**Table 4: Stimulation protocol and outcome.**

Stimulation protocol	No. of patients	Days of stimulation	Total Gn (IU)	Avg. oocyte yield	MII/total oocytes (% MII)	D2 Tr	D3 Tr	D5 Tr	Total Tr	Pregnancies (PR %)
<b>Agonist</b>	7	13	3315	3.2	13/23 (56)	2	1	0	3	1 (33)
<b>Antagonist</b>	15	12	3075	4	61/71 (85)	3	6	3	12	3 (25)
<b>Micro dose flare</b>	10	11	3100	4.9	31/49 (63)	4	2	1	7	1 (14)
<b>Stop protocol</b>	13	12	3150	5	55/65 (84)	5	5	3	13	2 (15)
<b>ANDRO-IVF protocol</b>	5	14	3215	2.6	7/13 (53)	1			1	1
	45				167/221 (75)	13	16	7	36	8

**Table 5: Stimulation protocol and embryology aspects.**

Stimulation protocol	No. of oocytes	2PN (fertilization rate) (%)	D2		D3		D5		Total transfer	Embryos vitrification		
			≤20% Frag	≥20% Frag	≤20% Frag	≥20% Frag	≤20% Frag	≥20% Frag		D2	D3	D5
<b>Agonist</b>	23	9 (39)	1	1	-	1	-	0	3	3	-	-
<b>Antagonist</b>	71	49 (69)	3	-	5	1	2	1	12	3	5	-
<b>Micro dose flare</b>	49	29 (59)	2	2	2	-	-	1	7	2	-	-
<b>Stop protocol</b>	65	49 (75)	4	1	4	1	2	1	13	2	-	2
<b>ANDRO IVF</b>	13	2 (13)	1	-	-	-	-	-	1	-	-	-
<b>Total</b>	221		13		16			7	36			

**Table 6: Adjuvant to treatment.**

Additives/adjuvant	No. of patients	MII/No. of oocytes retrieved (% mature oocyte)	D2 Tr	D3 Tr	D5 Tr	Total Tr
<b>GH</b>	12	29/43 (67)	2	7	-	9
<b>DHEA</b>	36	151/183 (82)	12	15	6	33
<b>Progesterone primed ovarian stimulation</b>	9	23/39 (58)	5	2	-	7
<b>Recombinant LH</b>	11	37/47 (78)	3	4	3	10
<b>Topical testosterone</b>	5	6/13 (46)	2	-	-	1

## DISCUSSION

Infertility is a social and family morbidity for couples. Advances in treatment have brought about hope and happiness for numerous couples with varied etiology of infertility. Ovarian reserve is a complex issue influenced by age, genetics and environmental factors. This depletion leads to reduced availability of gametes available for childbearing.<sup>5</sup>

In our cohort 12% of cases were found to have DOR, excluding those cases with exposure to cytotoxic drug or oncosurgery. Various ovarian stimulation protocols were used depending on patient profile. Of these 45 patients, 36 underwent embryo transfer. Three had fertilization failure and in other three embryo transfer was deferred due to thin

endometrium. In 3 of 45 stimulation was stopped due to poor response. Cycle cancellation in our study was defined as no follicular growth in spite of maximum stimulation for 6 days. 2 out of 3 cases of cycle cancellation in our cohort were in age related DOR and one was with idiopathic DOR. Jennifer in a study of 5,04,266 fresh IVF cycles over 8 years, saw a cycle cancellation rate proportional to severity of DOR, reflected by AMH and FSH titres.<sup>6</sup> All the three cases in our study group had AMH below 0.2. Out of 36 transfers, we got a pregnancy rate of 22%. Maximum rate was seen in ladies with surgical cause of DOR (33%). Bo Hyon in a similar study, with DOR defined as AMH below 1.1 ng/ml found that with personalized IVF-ET program high embryo transfer rate can be achieved.<sup>7</sup> We had an embryo transfer rate of approximate 70%. There were 3 cases of fertilization

failure. Two were following insemination and one was due to severe male factor and ICSI failure.

AMH is a dimeric glycoprotein belonging to group of transforming growth factor beta. AMH is most appealing and sensitive marker of ovarian reserve. Being stable in most phases of cycle it helps in guiding ovarian stimulation and also predicting IVF outcome.<sup>8,9</sup> Shunping found AMH to be better predictor of oocyte yield than FSH.<sup>10</sup> In our cohort AMH was directly proportional to oocyte yield, proportion of MII oocytes and pregnancy rate.

Mature oocytes percentage was 52% for AMH below 0.4, as compared to 80% for AMH above 1.1. Above AMH of 0.5 ng/ml, 58% of transfers were day 3 and beyond, giving a pregnancy rate of 33% compared to 10% pregnancy rate for all transfer below AMH 0.4 ng/ml. Oocyte quality is proportional to AMH titres in our study. More good quality embryos were available for transfer and further growth in AMH titres above 0.5 ng/ml. Similar to our study, Pierre lehmann found AMH titre below 0.4 ng/ml as predictor of poor prognosis. This can also guide about the outcome of IVF-ET cycle.<sup>11</sup>

With age there is a physiological decline in gametes. A finite number of oocytes are available for recruitment and growth during reproductive span of a lady. This is reflected by a raised FSH and visibly reduced AFC. Yajie Chang in a retrospective analysis of more than 4000 patients found young women with DOR to be in an ovarian hypo response stage.

In young patients with DOR, cleavage and blastulation rate are higher. This was also reflected in decreased abortion rate as compared to older DOR patients.<sup>12</sup> Beverley saw a decline in meiotic microtubule assembly and asymmetric division of genetic material in ageing embryos. The main dysfunction is in cohesion and telomere function. Dysfunction of these leads to aneuploidies and impairs cleavage.<sup>13</sup> Age remains a prognostic indicator for outcome of IVF-ET cycles. In our study of patients with DOR, we found a pregnancy rate of 21% for ladies below 40 years whereas only 1 patient of 13 conceived above age 40 years. Below age 35 years, 90% of oocyte had extruded the first polar body. Whereas mature MII, oocyte component was around 50% for ladies above 35 years. Inaki Gonzales also found age to negatively correlate with pregnancy rate ( $\leq 35$  years, 11% vs  $\geq 45$  years, 6%), miscarriage rate ( $\leq 35$  years, 7% vs  $\geq 45$  years, 50%) and ongoing pregnancy rate ( $\leq 35$  years, 10% vs  $\geq 45$  years, 3%).<sup>14</sup>

Age related decline in fertility may be because of increased incidence of aneuploidies. Even in morphologically normal embryos the potential may be compromised. More competent embryos with embryologically robust genetic material will reach day 5 growth.<sup>15</sup> In our study 35% of transfers (5 of 14 tr) were day 5 below the age of 30 years, as compared to 16% (2 of 15 tr) above the age of 35 years. ESHRE attempted to standardize definition of poor

ovarian reserve and increase the predictive potential of set of parameters. Consensus conference at Bologna finalized set of parameters to identify POR patients. Two of three were needed to classify a patient.<sup>16</sup> The criteria included age more than 40 years, poor AMH/AFC score or poor prior response to COH. Runa et al in a study of cohort of 459 ladies with different etiologies, for DOR found 84% had successful oocyte retrieval. Half of these underwent Embryo transfer. Clinical pregnancy rate was 35% in idiopathic DOR cases, 19% in age related DOR and 29% in surgical DOR cases. Cycle cancellation rate was similar in all groups.<sup>17</sup> In our cohort of patients, 5 of 21 (PR 23%) patients with idiopathic DOR conceived as compared to 11% PR in age related DOR. Maximum pregnancy rate of 33% was seen in patients with surgical cause of DOR. Similar to this study we found lowest PR in age related DOR. 2 of 6 patients with surgical cause of DOR conceived. Boh Yon in a similar analysis of patients of DOR found maximum fertilization and PR in patients with previous ovarian surgery as compared to idiopathic or cytotoxic chemotherapy induced DOR.<sup>18</sup> Excision of endometrioma is the most common reason for ovarian surgery. Thermal energy sources lead to tissue destruction and depletion of available pool of follicles. Local inflammation and auto immune reaction may contribute to DOR in endometrioma patients.<sup>17</sup> Ismail Guler et al found ovarian reserve tests, number of oocytes retrieved, percentage of MII oocytes, cleavage rate and blastulation rate to be lower in endometrioma operated group as compared to non-operated group. In our study ladies with previous surgery with diminished ovarian reserve, average oocyte yield was 9. Of these 82% were mature MII oocytes. 6 of 7 patients underwent embryo transfer, giving a pregnancy rate of 33%. Five of seven ladies with prior surgery had undergone excision of endometrioma. Two were operated for simple ovarian cyst. Except two, all were operated laparoscopically.<sup>19</sup>

Agonist protocols are expected to give a higher yield of mature oocyte. This is because of more synchronized stimulation. Without gonadotropin suppression a cohort of follicles may show an exaggerated response leading to non-uniform cohort of growing follicles. Agonist protocols have lesser cycle cancellation rate.<sup>20</sup> In our study we got a retrieval rate of 56% MII mature oocyte with fertilization rate of 69%. There were no cycle cancellations. Average cycle oocyte recovery was 3.9. Ming et al found an implantation rate of 25% for agonist cycle as compared to 10% for antagonist protocol IVF in a comparative study of 577 patients with DOR.

Feiyan Zhao also found a higher implantation rate of 25% for agonist cycle. (18% for antagonist cycle). Also, these pregnancies were likely to be healthier reflected by lower abortion rate (28% vs 34% in antagonist cycles).<sup>21</sup> Similarly, in our study cohort we found a beta HCG positive rate (Implantation Rate) 33% for agonist cycle as compared to 25% for antagonist cycle. Although we had no cycle cancellation, agonist protocol is likely to have more cycle cancellation as compared to antagonist cycle.

Antagonist protocol has a shorter period of stimulation, thus proves to be less costly. Ming et al also found that average gonadotropin dose required for Antagonist cycle to be around 3018 IU as compared to 3298 IU for agonist cycles.<sup>22</sup> Rong Yu in a comparative study of different protocols for COH, also found lower total dose and duration of gonadotropin consumption in antagonist cycles. It might be because of lesser pituitary inhibition as compared to agonist protocol.<sup>23</sup> In our study also we found total dose of gonadotropin and duration to be lesser as compared to agonist protocol and androgen-based protocols. In our study population we found antagonist protocol on an average required 12 days of stimulation with an average oocyte yield of 4 oocytes. Mature oocytes percentage was 85%. 75% of transfer were day 3 and beyond. 77% (7 of 9) of these had no fragmentation or below 20% attesting to their good quality. Fertilization rate was more than 80% and we had maximum vitrification in this category of stimulation protocol.

The economic burden on couples undergoing the ART treatment is huge. Direct expenditure on drugs, consumables, consultation and procedures are also compounded by indirect expenses. They include loss of wages, absenteeism and transportation, directly affecting finances. Miaomiao in a retrospective study of 1638 patients found cost of antagonist cycle to be lower than agonist cycle for fresh ET, (\$1600 for antagonist vs 1900 for agonist).<sup>24</sup> Toftager et al found antagonist protocol more patients friendly. Episodes of emotional lability, unexpected crying and quality of sleep better in antagonist cycles.<sup>25</sup> Raoul found increased number of follicles, higher number of mature oocytes and a greater number of top-quality embryos in stop Antagonist protocol. This may be because of better suppression of premature LH surge.<sup>26</sup> More synchronous growth of embryos leads to better outcome of IVF cycle. Stop protocol is adapted to avoid premature LH surge. The agonist down regulation is discontinued and stimulation started. It gave a mature oocyte yield of 84%. More number of blastocysts was available for transfer. Because of a greater number of good quality oocyte, vitrification more embryos were available. Thus, also increasing cumulative pregnancy rate.

Microdose protocol uses the stored pituitary FSH and LH. Agonist triggers a release of gonadotropin which is used as a flare to initiate a surge in growth of primordial follicles. This initial spurt of growth is aided by Gonadotropin to continue with the initial growth.<sup>27</sup>

ANDRO IVF protocol is based on the concept that androgens are required for recruitment of follicles. As per two cell theory they act as a substrate for theca cell to increase FSH receptors. Increase in receptor concentration increases the responsiveness of growing pool of primordial follicle to stimulation.<sup>28</sup> This protocol was especially useful with resistant ovary with previous failure in stimulation. We could retrieve oocyte cumulus complex in 4 of 5 patients. 7 of 13 oocyte were mature and lead to one pregnancy. DOR is relatively androgen deficient stage.

Androgens increase the FSH receptor expression and acts as a substrate for theca cells. Supplementation by DHEA and testosterone leads to improved recruitment of preantral and antral follicles. This improves the oocyte yield, especially in ladies with previous cycle cancellation. Testosterone improves the cycle outcome.<sup>29</sup> In our study, 5 patients yielded 53% MII oocytes. After prolonged stimulation one, day 2 transfer was done which resulted in a positive beta HCG titre.

Ya yu et al in a study of more than 300 patients found that embryo quality was better and more embryos were available for vitrification. Duration and dose of gonadotropin required was lower in flare supplemented antagonist protocol.<sup>30</sup> In our study flare antagonist cycles averaged 11 days of stimulation. Stop and antagonist cycles averaged 12 days of stimulation. Lowest cumulative dose of Gonadotropin was used in flare antagonist cycle. ANDRO cycles were the longest in stimulation. But these had previous cycle cancellation and AMH in the range of POF. This study also found that in antagonist protocol more numbers of MII oocytes were retrieved. Cleavage rate and quality of embryos were definitely better. Similarly, in our study a greater number of embryos was available for vitrification.

In antagonist, stop protocol and microdose protocol we used dual trigger. Gonadotropin agonist was supplemented with injection HCG. This was aimed to rescue and augment luteal function. Agonist trigger alone needs to be supplemented with more intense progesterone supplementation or need to intermittently give reduced dose of injection HCG. In our cohort, addition of dual trigger in the form of injection HCG along with agonist gave more than 80% MII oocyte. Agonist binds to GnRH receptors more avidly and improves the decidualisation of endometrium. Agonists activate urokinase type plasminogen activator thus improving endometrial receptivity.<sup>31</sup> San-Nung Chen et al saw a DHEA supplementation for 10-12 weeks before IVF yields a higher number of oocytes and better quality embryo. There is improvement in cumulative pregnancy rate also. Androgen supplementation improves follicular recruitment, steroidogenesis, and increases the number of primary and preantral follicles.<sup>33</sup> In our study similar supplementation gave 82% mature MII oocytes. There was more day 5 vitrification. Overall quality of embryos was good.

Endometrial preparation is a challenging step in cases of DOR. We used tablet Progynova, Intrauterine G-CSF, Ecosprin and Sildenafil citrate. Sildenafil citrate is a phosphodiesterase inhibitor. Due to augmented effect of NO on sub endometrial blood flow it improves proliferation of endometrial cells. Sildenafil also has a positive effect on oocyte quality.<sup>34</sup> Injection G-CSF is a glycoprotein with growth factor and cytokine function. It augments the C-AMP mediated decidualisation of endometrial stromal cells. Thus, leading to expansion of receptive endometrial pattern.<sup>35</sup> We used a dose of 300

mcg instilled by an IUI catheter. Growth hormone is a peptide hormone produced by anterior pituitary. Receptors are present in uterus, tubes, ovary and endometrium. Improvement in endometrial receptivity is due to proliferative effect on endometrial stromal cells. We used injection GH in a dose of 4 IU from the start of GN stimulation. Higher doses of GH are also proposed to reduce the total dose of gonadotropin required.<sup>36</sup> Yi Xuan LI in a study of 184 patients with POR saw a higher number of mature oocyte and top-quality embryos in GH supplemented group. The clinical pregnancy rate was 31% as compared to 16% in non-GH group.<sup>37</sup> In our study for 12 GH supplemented patients, we got 29 mature oocytes. (67%) Maximum number of embryos reached 8 cell stages with minimal fragmentation. Qun Guan et al found co administration of Ecosprin and GH significantly improves oocytes yield, maturation of oocytes and fertilization rate.<sup>38</sup>

Chun mi yun et al found progesterone primed ovarian stimulation to give better oocyte yield. It more efficiently suppresses premature LH surge.<sup>39</sup> In our study population we found MII oocyte yield to be 58%. There was minimal cycle cancellation due to poor embryo growth. 7 of 9 patients underwent ET, five on day 2 and two on day 3.

Recombinant LH supplementation is based on two cell two gonadotropin theory. LH helps conversion of Cholesterol to androgen. This increased intraovarian androgen helps recruitment of preantral and antral follicles. LH increases FSH receptor expression in granulosa cells and synergizes IGF I mediated synchronous growth of follicles. R-LH is recommended for ladies with previous poor response and advanced reproductive age.<sup>40</sup> In our study of 11 patients we got 78% mature oocyte. Embryo quality was attested by 3 transfers done on day 5. Only one patient of 11 could not undergo an ET due to post fertilization arrest of cleavage.

### Limitations

The study of obstetric follow up and outcome of ladies conceived with various protocols in fresh and vitrification cycles were beyond the scope of present study. A larger cohort cycle would have given more statistically significant results.

### CONCLUSION

With personalized stimulation protocol and care ladies with DOR may have acceptable outcomes of ART treatment. Age and AMH remains tool for prognosis and counselling about treatment outcome.

Various protocols have their own benefits and disadvantage and need to be applied depending on clinical profile of the patient. Antagonist protocols are shorter in duration with lesser total gonadotropin consumption, thus more economical. Flare and agonist-stop protocol are effective alternates. Adjuvant to treatment improves the

cycle outcome. Growth hormone, androgen and estrogen are important additive to various aspect of ART cycle.

Inspite of being a grim and challenging situation, outcome of DOR cases may be improved with multipronged strategy.

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