Revised Super-long Down-regulation Protocol Improves the Outcome of Infertile Patients with Repeated Implantation Failure in IVF/ICSI-ET

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Objective  To investigate the outcome of revised super-long down-regulation protocol (RSDP) for in vitro fertilization / intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) in the special infertile patients with repeated implantation failure (RIF).

Methods  Patients with RIF were divided into RSDP group and routine long down-regulation protocol (RLDP) group. In RSDP group, gonadotropin releasing hormone agonist (GnRHa) was injected intramuscularly by 2.5 mg in mid-luteal phase for the first time and 1.25 mg after 28 d; gonadotropin (Gn) was started 14 d later after the second GnRHa dose. IVF/ICSI-ET was performed according to the routine procedure. The clinical outcomes of RSDP group were compared with those of RLDP group.

Results  In RSDP group, the number of retrieved oocytes and valid embryos was significantly lower (P<0.05); there were no significant differences about fertilization rate (P>0.05); both good-quality embryo rate and implantation rate were significantly increased (P<0.005); clinical pregnancy rate was obviously improved (P<0.05), as compared with RLDP group.

Conclusion  RSDP can improve the IVF outcomes significantly in RIF patients.

Key words: implantation failure; revised super-long down-regulation protocol (RSDP); routine long protocol; in vitro fertilization (IVF)
Now, routine long down-regulation protocol (RLDP) and other controlled ovarian hyperstimulation (COH) protocol have been commonly applied to infertile patients for treatment of \textit{in vitro} fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) and got high pregnancy rate, but there were still some special patients with repeated implantation failure (RIF). RIF was determined when transferred embryos failed to implant after several IVF treatment attempts\cite{1}. However, there were no formal criteria defining the number of failed cycles or the total number of embryos transferred in IVF attempts. The incidence rate of RIF was about 10%. As a highly coordinated event, successful embryo implantation mainly depends on well-functioning endometrium as well as excellent quality embryos. How to improve the clinical pregnancy rates of these special patients was one of the tough problems for IVF. Since good COH protocol could result in multiple synchronized developed follicles and high quality mature oocytes, and improve fertilization rate and pregnancy rate, we applied revised super-long down-regulation protocol (RSDP) to these patients in our centre, and IVF outcomes were compared between RSDP and the control group of RLDP. To our knowledge, this is the first comparison of these two protocols in RIF population.

**Materials & Methods**

**General data**

In our centre, RIF was defined as the absence of a gestational sac on ultrasound at $\geq 5$ weeks post-embryo transfer after $\geq 3$ embryo transfers with high-quality embryos or the transfer of $\geq 10$ embryos in multiple transfers\cite{2}. According to this criterion, a total of 112 patients with RIF in our centre from January 2010 to November 2013, were divided into RSDP group and RLDP group. Hysteroscopy was routinely performed in all of these RIF patients to assess the uterine cavity and have treatment accordingly. The age, reasons and duration of infertility, basal endocrine parameters and the means of assisted conception were compared between the two groups.

**Methods**

**Revised super-long down-regulation protocol**

GnRHa (long-acting triptorelin, Beaufour Ipsen Pharmaceutical Co. Ltd) 2.5 mg was injected intramuscularly 7 d after ovulation or in the 16th day of the oral contraceptive cycle with Diane 35 (Ethinylestradiol and Cyproterone Acetate Tablets, Schering GmbH & Co. Produktions KG), and repeated 28 d later with the dose of 1.25 mg. Follicle stimulating hormone (FSH, Gonal-F, Serono) was used for follicle development 14 d later.

**Routine long down-regulation protocol**

GnRHa 1.25 mg was injected intramuscularly once at mid-luteal phase for down-regulation or in the 16th day of the oral contraceptive cycle with Diane 35. FSH was used for
COH 14 d later.

**Evaluation of the follicle development**

Ultrasonograph combined with serum hormone measurement was used for evaluation of the follicle development. Recombinant human chorionic gonadotropin (Merck Serono) 0.25 mg was injected to promote the maturation of the oocytes when at least one of the dominant follicle reached 18 mm in diameter or two with 17 mm in diameter. Oocytes were retrieved after 34–36 h. The culture of oocytes and embryos, fertilization, ICSI, and embryo transfer were performed according to the standard protocols. The embryos were transferred at 4–8 cells and 1–3 embryos were transferred. Clinical pregnancy was confirmed by elevated serum hCG level and pregnancy sac identified by ultrasonography.

**Statistical analysis**

Data were expressed as mean ± standard deviation (x ± s), or percentage (%). SPSS 10.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. T test and chi-square were used respectively. P<0.05 was deemed statistically significant.

**Results**

The characteristics of patients are shown in Table 1. Ten cycles were cancelled in RSDP group and RLDP group respectively for failed oocytes retrieval, unfertilization, or cleavage failure. The reasons for cycle cancellation are shown in Table 2. The ET cycles in RSDP group and RLDP group were 42 and 50, respectively. As for category, reasons, and duration of infertility, body mass index (BMI), basal E2 and the ratio of IVF/ICSI cycles, there were no significant differences between the two groups (P>0.05). But the patients’

<table>
<thead>
<tr>
<th>Item</th>
<th>RSDP group</th>
<th>RLDP group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary infertile (n)</td>
<td>30</td>
<td>36</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Secondary infertile (n)</td>
<td>22</td>
<td>24</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Infertile duration (year)</td>
<td>5.8 ± 3.0</td>
<td>4.8 ± 2.9</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Age (year)</td>
<td>33.8 ± 5.1</td>
<td>29.6 ± 4.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>22.0 ± 3.2</td>
<td>22.3 ± 3.5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Basal E2 (pg/ml)</td>
<td>56.8 ± 30.2</td>
<td>57.0 ± 32.6</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Basel FSH (IU/ml)</td>
<td>8.0 ± 1.8</td>
<td>6.8 ± 2.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
<td></td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Male factor (%)</td>
<td>9.5</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>Tubal factor (%)</td>
<td>57.1</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>Male factor + tubal factor (%)</td>
<td>14.3</td>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td>Ovulation failure (%)</td>
<td>0.0</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Endometriosis (%)</td>
<td>4.8</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Idiopathic (%)</td>
<td>14.3</td>
<td>16.0</td>
<td></td>
</tr>
</tbody>
</table>
average age in RSDP group was older than that in RLDP group by about 4 years with significant difference \((P<0.05)\), and the basal FSH level was higher in RSDP group than in RLDP group \((P<0.05)\).

As shown in Table 3, the Gn dosage had no significant difference between the two groups \((P>0.05)\), but Gn duration in RSDP group was significantly shorter than that in RLDP group. The \(E_2\), LH and P levels on hCG injection day were lower in RSDP group than in RLDP group with statistical significance. The number of retrieved oocytes and mature oocytes in the RSDP group was significantly lower \((P<0.05)\), as compared with those in RLDP group, but there was no statistical difference for fertilization rate \((P>0.05)\). Moreover, both the good-quality embryos rate and implantation rate were significantly higher in RSDP group as compared with those in RLDP group \((P<0.05)\), regardless of the significantly lower valid embryos in the former \((P<0.05)\). More clinical pregnancies with fetal heart activity were achieved in RSDP group than that in RLDP group \((P<0.05)\).

**Discussion**

Routine down regulation regime had been commonly used for most infertile women. Pituitary down regulation by GnRHa injection at mid-luteal phase could effectively inhibit endogenous and premature LH surge and prevent the premature luteinization of oocytes. But the pregnancy rate resulted from RLDP and other various protocols were not satisfied for patients with repeated implantation failure. Women who experienced repeated IVF failure with implantation barrier presented a challenging and frustrating problem. It was well accepted that successful embryo implantation depended on the two key factors of embryo quality and endometrium receptivity. So improvement on embryo quality and endometrium receptivity would lead to enhancement in implantation rate and pregnancy rate. Various treatment protocols have been proposed targeting at this particular cohort of women now. These protocols can be reviewed as followed:

1) Hysteroscopy performed to find and treat RIF anatomic causes.
2) The application of oral or vaginal estrogen pills, aspirin, and other medications that may increase blood flow to the endometrium.

3) Mechanical endometrial stimulation performed in the cycle preceding the actual treatment cycle to induce an inflammatory response that may facilitate the preparation of the endometrium for implantation [3-5].

4) Locally or systemically administered human leukemia inhibitory factor or granulocyte colony-stimulating factor to improve IVF outcome in patients with unexplained RIF [6-8].

5) Treatment with low molecular weight heparin (LMWH) or a mini-dose of aspirin and/or corticosteroids for thrombophilia and connective tissue disease [9,10].


7) Intracytoplasmic morphologically selected sperm injection (IMSI) for those with a high percentage of abnormal sperm cells [12,13].

8) Measures to ameliorate the embryonic factors such as zygote intrafallopian transfer [14], blastocyst transfer, sequential embryo transfer [15], and embryo coculture [16].

9) Preimplantation genetic diagnosis (PGD) for genetic causes.

Since protocol of COH played pivotal role in the improvement of embryo quality and implantation, and pregnancy rate of the routine protocols was not satisfying in patients with
RIF, we used RSDP on these patients and demonstrated that pregnancy rate of RSDP was much higher than that of RLDP at the same period of time. Although the number of retrieved oocytes and valid embryos was significantly lower in RSDP than in RLDP, the good-quality embryos rate and implantation rate both were significantly higher in the former than those in the latter. The levels of E\textsubscript{2}, LH and P on hCG injection day in RSDP was lower than those in RLDP especially with statistical significance. We speculated that less follicles in the cohort were recruited by RSDP resulting that lower levels of estradiol were produced and less oocytes and embryos were achieved. These results don’t agree with those of Zhang Hui-juan\textsuperscript{[17]} who compared between the two protocols in patients with poor response, mainly because their RSDP and the according subjects were completely different from ours. In our results, RSDP attained to the satisfying pregnancy rate of 57.1%, as was much higher than that of RLDP with the 32% pregnancy rate although the patients’ average age was elder by about 4 years in RSDP group compared with RLDP group.

We presumed the reasons for the improvement of pregnancy rate in RIF patients were as followed. Firstly, the internal milieu was changed to enhance the endometrium receptivity. RSDP management was similar to that of hypogonadotrophism, and it helped to inhibit irregular hyperplasia and inflammation of endometrium to be in favour of synchronization between follicle development and endometrium. Moreover, the abnormal internal environment caused by endometriosis could also be significantly inhibited, since the application of GnRHa in endometriosis patients could not only relieve the symptoms, but also inhibit the endometriosis lesion and improve the pelvic and ovary microenvironment, leading to amelioration of reproductive capacity. Secondly, RSDP could significantly improve the quality of embryos. Higher excellent embryo rate was obtained despite the low oocytes and embryos achievement in RSDP. It was obvious that injection of GnRHa could inhibit the hypothalamic-pituitary-ovarian (H-P-O) axis and the secretion of LH leading to the decrease of androgen, E\textsubscript{2} and P production and secretion. The decrease of androgen level might reduce the adverse effects on the follicle development and avoid the excessive recruitment of follicles, especially in PCOS\textsuperscript{[18]}. In addition, the relatively low P level on hCG injection day might ameliorate the receptivity of the endometria and improve the pregnancy rates. In the process of COH, the effect of LH level at follicular phase on the outcome of ART was not confirmed. It is generally accepted that excessive LH at follicular phase or premature LH surge might cause abnormal development of follicles and inhibition of the secretion of meiosis inhibition factor (MIF) by oocytes, and then cause the failure of fertilization because of over-maturation of the oocytes. Besides, the high level of LH could induce the elevation of estrogen and the P receptor appeared prematurely on the endometrium. The premature transformation of the endometria to secretory phase had adverse effect on embryo implantation\textsuperscript{[19]}. So well-controlled LH and P levels on hCG injection day would contribute to the improvement of pregnancy rates. We
supposed that RSDP could provide complete pituitary down-regulation and concordant endocrinial environment for follicular development, as leading to the high excellent embryo rate in these RIF patients.

In RSDP, the gonadotropin dosage was not increased but the duration of medication was decreased significantly compared with RLDP, which proved that RSDP was economical of time and money.

It is necessary to clarify the ideal cycle number of GnRHa pretreatment and how RSDP improved the outcome of IVF-ET in infertile patients. Super-long down-regulation protocol had been routinely applied in presently, and multiple investigations supported that the application of GnRHa for 3–6 cycles before IVF was necessary. Ma et al.\(^{[20]}\) analyzed the IVF outcome of endometriosis patients who underwent 2–3 cycles of 3.75 mg GnRHa before IVF and found that the extended pretreatment of GnRHa would increase Gn dosage and extend duration of COH although pregnancy rate was increased. It was discord with our results. In our study, twice injection of small dose of GnRHa before the COH obtained the remarkable clinical pregnancy rate of 57.1%. The reasons might be that we only use small dosage of GnRHa, the over-depression of the H-P-O axis had been evaded, and the poor response to COH had been also prevented.

In a word, the application of RSDP in patients of RIF would not only increase the pregnancy rates, but also decrease the cost. We might consider this regimen as an effective and economical assisted conception protocol. But the limited number in our study might cause bias and a further large scale investigation is necessary.

References


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