Comparison of different stimulation protocols efficacy in poor responders undergoing IVF: a retrospective study

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<td>Complete List of Authors:</td>
<td>Madani, Tahereh; Royan, Endocrinology and Female Infertility Department Ashrafi, Mahnaz; Royan, Endocrinology and Female Infertility Department; Faculty of Medicine, Iran University of Medical Science, Obstetrics and Gynecology Department Mohammadi Yeganeh, Ladan; Royan, Endocrinology and Female Infertility Department</td>
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<tr>
<td>Keywords:</td>
<td>GnRH agonist, GnRH antagonist, IVF, poor responder</td>
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</table>
Dear Sir,

Please be informed that all amendments regarding the manuscript titled “Comparison of different stimulation protocols efficacy in poor responders undergoing IVF: a retrospective study” were performed according to the reviewers comments.

All the items modified as per the following and underlined in the text:

The first reviewer:

1) Definition of cycle cancellation: (page 6)
   Cycle cancellation was recommended when fewer than 2 growing follicles were noted.
   Meanwhile, cancellation rate was calculated as:
   \[
   \frac{\text{Number of cancelled cycles}}{\text{total number of cycles}} \times 100
   \]

2) Table II was amended to clear the ambiguities regarding the significant differences between groups.

3) As mentioned in the materials & methods section, in this study we considered at least 2 criteria for poor respond women selection and according to our definition, age cannot be considered as the only definite criterion. However, this item was explained in discussion part as a limitation of the study (page 11, discussion section).

4) In this study, high quality embryos (Grade A&B) were selected to transfer. Therefore, male factor wouldn’t be effect on IVF outcomes.

The second reviewer

1) In this study, for data that were not normally distributed (number of follicles>18mm on hCG day), a Kruskal-Wallis test was used to compare the mean values between the stimulation protocols (page 6).

2) As your suggestion, we substituted Mean±SD instead of SEM for describing the characteristics and results of different groups (table I&II).

3) Power analysis was performed by chi-square test and NCSS software 2007 and revealed that the power for main outcomes such as clinical pregnancy, implantation and cancellation rates were 0.88, 0.75 and 0.81 respectively.

4) The required revisions in introduction result and discussion parts were done and some information were added for improvement the contents of discussion section.

Should you need more requirements, please do not hesitate to contact me.

Sincerely yours

Dr. Tahereh Madani
Comparison of different stimulation protocols efficacy in poor responders undergoing IVF:

a retrospective study

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Running Title
Different stimulation protocols in poor responders

Key words: GnRH agonist; GnRH antagonist; IVF; poor responder
Abstract

Purpose to compare the efficacy of different stimulation protocols on pregnancy outcomes in poor responders undergoing IVF.

Materials and Methods This was a retrospective study to compare the efficacy of four different protocols including GnRH agonist (long, short, miniflare) and GnRH antagonist on pregnancy outcomes in poor responders. This investigation was performed on 566 poor respond patients who were candidates for IVF. Main outcome measures included the total number of oocytes and mature oocytes retrieved, pregnancy rates, implantation and overall cancellation rates which were compared between four mentioned groups.

Results Number of follicles>18mm on hCG day were significantly higher in GnRH-a long versus GnRH antagonist, GnRH-a short and GnRH-a miniflare protocols. The mean number of oocytes and mature oocytes retrieved were significantly higher in GnRH-a long versus miniflare (4.7±3.05 vs 3.26±2.9 and 3.69±3.1 vs 2.65±2.2 respectively). There were no significant differences in implantation, pregnancy and overall cancellation rates between four groups.

Conclusion The present study suggests that the application of four different protocols in poor respond patients seem to have similar efficacy in improving clinical outcomes such as implantation, pregnancy rates and cancellation rate even though GnRH-a long protocol yielded more retrieved oocytes and mature oocytes compared to GnRH-a miniflare protocol.
Introduction

Despite the recent advances in ovarian stimulation protocols, there are still women who respond poorly to gonadotropin stimulation and are classified as poor responders[1]. Poor respond to controlled ovarian hyper stimulation is one of the greatest challenges in assisted reproduction which may occur in 9-24% of the women undergoing IVF [2]. Although different criteria have been used to define the poor responders, they are traditionally identified as women at the end of the reproductive years with reduction of the ovarian reserve[3]. However, age is not the only definite reason and other factors such as previous ovarian surgery [4], pelvic adhesions [5] and high body mass index[6] may be associated with poor ovarian response.

Several stimulation protocols have been proposed to improve ART outcomes in poor responders. These treatments include variations in the type and administration timing of gonadotropins or GnRH analogues, increasing the dose of gonadotropins, using GnRH-a long protocol and flare regimens, using GnRH antagonist plus recombinant luteinizing hormone, administration of aromatase inhibitors, corticosteroids, Growth hormone or Aspirin as adjuvant therapies [1, 7-11]. Most of these interventions have met low success and there is still high cycle cancellation and pregnancy failure due to the low number of oocytes and embryos with poor quality[1].

During the past years, several studies on the treatment regimens in poor responders with different kinds of protocols have been performed [12-17]. However, despite various accomplished studies, the matter for which protocol is preferable in poor responders remains controversial. Thus in this retrospective study, we aimed to compare the efficacy of four different stimulation protocols on pregnancy outcomes in poor respond patients.
Methods

Subjects

This investigation is a retrospective study including 566 patients who were undergoing IVF in Royan infertility center, Tehran, Iran, from April 2007 to January 2009 and were considered as poor responders.

Patients with only one ovary were excluded from the study. Poor responders were defined when at least two of the following criteria were present:

1- age>40      2- antral follicles<5      3- Basal FSH>12mIU/ml      4- prior history of poor response to controlled ovarian hyperstimulation (peak E2<500pg/ml and/or ≤3 oocytes retrieved)
5- prior cycle cancellation

Protocols

In this study, all patients had pre cycle ovarian reserve testing which included an assessment of basal serum FSH level and antral follicle count in the early follicular phase and criteria evaluation for classification as poor responders.

In GnRH-a long protocol, patients received low dose oral contraceptive pills from the 5th day of the preceding menstrual period and followed by GnRH-a (Superfact,Aventis,Frankfurt,Germany), 500μg subcutaneous, started from the 21st day of that cycle. Once the suppression of the ovaries was confirmed (LH<5mIU/ml, Estradiol<50pg/ml), gonadotropin administration was started. In GnRH antagonist protocol, the patients received oral contraceptive pills from the previous cycle for 21 days followed by exogenous gonadotropins
administered from the 2nd or 3rd day of the next cycle and later 0.25 mg Cetrorelix (Cetrotide, Serono, Geneva, Switzerland), was administered daily, once the leading follicle reached 14 mm in diameter.

In GnRH-a short protocol, GnRH-agonist (Superfact) was initiated from the third day of menstruation cycle and in the next day gonadotropin was administered. In GnRH-a miniflare protocol, the patients received 80 µg/d GnRH-agonist (Superfact) from the second day of menstruation cycle and gonadotropin administration began from the next day.

For all protocols, gonadotropin dosage was tailored according to individual ovarian response. Administration of gonadotropins, GnRH agonist and antagonist were continued until the day of hCG administration. Final oocyte maturation and ovulation induction were obtained by using 10,000 IU of urinary hCG (Pregnyl, Organon, Netherland) or 250 µg recombinant hCG or 500 µg recombinant hCG (Ovitrelle, Serono, Italia) in the presence of at least two follicles measuring >18 mm in diameter. Oocyte retrieval was performed 35-36 hours after hCG administration and embryos were transferred 2-3 days thereafter.

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The ovarian stimulation characteristics, number of follicles >18 mm, number of oocytes retrieved and number of mature oocytes, number of embryos transferred, pregnancy rates, implantation rate and overall cancellation rate were assessed and compared between four groups.

Cycle cancellation was defined when fewer than two growing follicles of an appropriate growth pattern were noted. Cancellation rate was calculated by dividing the number of cancelled cycles by the total number of cycles (%).
Statistical analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS) version 16.0. For normally distributed data, analysis of variance (ANOVA) was used to compare the mean values between different stimulation protocols. For data that were not normally distributed (number of follicles>18mm on hCG day), a Kruskal-Wallis test was used to compare the mean values between the stimulation protocols. The chi-square test was used to compare implantation rate, chemical and clinical pregnancy rates and overall cancellation rate among four groups. A P value of <.05 was considered statistically significant.

Results

In this retrospective study, the efficacy of four different protocols consists of GnRH antagonist and GnRH-a (short, long and miniflare) on pregnancy outcomes in 566 poor responders were evaluated. All protocols were comparable regarding the mean body mass index and duration of infertility but age was significant in long versus short and long versus miniflare protocol. A two way ANOVA analysis was performed to assess the effect of age on the clinical results which was significant for the number of retrieved and mature oocytes. The patients' characteristics of the poor respond women are shown in table I.

There were significant differences in duration and doses of gonadotropins required. Number of follicles>18mm on hCG day were significantly higher in long versus antagonist (P=0.026), long versus short (P=0.002) and long versus miniflare (P<0.001). The mean number of oocytes and mature oocytes retrieved were significantly higher in long versus miniflare(4.7±3.05 vs 3.26±2.9 and 3.69±3.1 vs 2.65±2.2 respectively).
On the other hand, the four groups were statistically similar with regard to all other compared parameters such as size of dominant follicle, endometrial thickness on hCG day and puncture day, number of embryos transferred, implantation rate, pregnancy rates and overall cancellation rate. The details of four groups are summarized in Table II.

Power analysis was performed by chi-square test and NCSS software 2007 and revealed that the power for main outcomes such as clinical pregnancy, implantation and cancellation rates were 0.88, 0.75 and 0.81 respectively.

**Discussion:**

In this study, we have evaluated four different types of protocols in a large series of poor respond women undergoing IVF and to the best of our knowledge, this is the first study made on this kind. Results of the current study demonstrate that there were significant differences in the number of retrieved oocytes and number of mature oocytes in the long versus miniflare protocol.

Weissman et al. [18] in a prospective randomized trial compared two protocols in a group of 60 poor responders in form of that one group received a modified flare protocol and the second group received a modified long protocol. Significantly, more oocytes were obtained in a modified GnRH-a long protocol in comparison to a modified GnRH-a flare protocol.

According to our findings, the use of ovarian protocols have similar IVF outcomes in terms of implantation rate, chemical and clinical pregnancy rates and overall cancellation rate in poor respond women. Some other studies comparing different stimulation protocols in poor responders similarly showed no significant differences between the clinical results of stimulation regimens.
Akman et al.[19] compared two stimulation regimens consist of GnRH agonist flare with multiple doses GnRH antagonist on 48 poor respond patients and demonstrated similar clinical outcomes. In a later study by Mohamed et al.[17] GnRH agonist flare were compared with GnRH antagonist protocol and concluded that the two protocols were comparable in terms of pregnancy rates. Similarly, in other more recent studies, no significant differences were found between different types of protocols in poor responders to ovarian stimulation [13, 20, 21].

It is noteworthy that, the advantages of the pituitary down regulation by GnRH agonists were demonstrated in many trials of patients who had failed to respond to gonadotropin alone and generally showed improved outcomes such as decreasing cancellation rates and improved pregnancy rates [22, 23].

GnRH agonist long protocol is the mostly used protocol worldwide. However, the reduced sensitivity to gonadotropins with administration of GnRH agonist long protocol has led some specialists to reduce the dose and duration of agonist in poor respond patients.

Many investigators consider the GnRH-a flare protocol to be the protocol of choice for low responders [24-26]. It is believed that initiation of GnRH-a therapy in early follicular phase has advantage of the endogenous gonadotropin flare effect [23].

GnRH-antagonists have recently been introduced as a treatment strategy for poor responders and have been proven to be effective in prevention of premature LH surge without suppressing early follicular development [19, 27]. Some studies concluded that GnRH antagonist protocols have some advantages beyond other poor responder protocols. They noted that, GnRH antagonists may shorten the duration of stimulation and reduce the total gonadotropin requirements and patient costs [28-30]. Similarly, the use of GnRH-antagonist in our study population resulted to reduction in duration of stimulation and gonadotropin usage compared to GnRH-agonist long
protocol which the difference was significant for duration of stimulation. However, high gonadotropin requirement is a normal phenomenon in poor respond patients.

Our study has some obvious limitations in terms of its retrospective nature and significant age between groups. Our data analysis revealed that poor responders under the age of 38 years have more retrieved and mature oocytes than their peers over 38 years but no relationship between age and other clinical outcomes such as pregnancy, implantation and cancellation rates were achieved. Therefore, superior results in terms of retrieved oocytes and mature oocytes in long protocol group might be due to younger age in this group of patients. However, in a study with retrospective design, managing the similar patients' characteristics between groups cannot be guaranteed.

One of the most common problems faced in poor responder studies is no existence of a unique global accepted definition of the poor responders and a variety of criteria have been used in studies[10]. In our study, different criteria used for selection of poor responders have led to a heterogeneous study population. However, the results might differ with the application of more strict and unique criteria for this group of patients.

Ultimately, the present study demonstrates that the application of four different protocols in poor respond patients seems to have similar efficacy in improving clinical outcomes such as implantation and pregnancy rates, eventhough GnRH-a long protocol yielded more retrieved oocytes and mature oocytes compared to GnRH-a miniflare protocol.

There are insufficient evidences to support the definite intervention in the management of poor responders undergoing IVF. Thus, further prospective trials in more homogenous population
would be necessary to determine the optimum COH protocol to improve the outcomes of this patient population.

**Declaration of interest**

The authors report no declarations of interest.
Table I. Patient characteristics

<table>
<thead>
<tr>
<th>characteristics</th>
<th>Antagonist</th>
<th>Short</th>
<th>Long</th>
<th>Miniflare</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cycles</td>
<td>72</td>
<td>73</td>
<td>136</td>
<td>285</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37.5±5.15</td>
<td>39.19±4.38</td>
<td>35.58±6</td>
<td>38.06±5.18</td>
<td>&lt;.01</td>
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<tr>
<td>Duration of infertility (years)</td>
<td>10.31±6.7</td>
<td>11.86±6.6</td>
<td>10.59±6.66</td>
<td>10.65±6.97</td>
<td>NS</td>
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<tr>
<td>Body mass index</td>
<td>23.5±9.51</td>
<td>22.8±10.03</td>
<td>24.2±8.65</td>
<td>23.7±9.43</td>
<td>NS</td>
</tr>
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</table>

Notes: Data are expressed as mean±SD
NS, not statistically significant
Table II. Cycle characteristics and clinical outcomes

<table>
<thead>
<tr>
<th></th>
<th>Antagonist</th>
<th>Short</th>
<th>Long</th>
<th>Miniflare</th>
<th>Pvalue</th>
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<tr>
<td>Number of gonadotropin ampoules</td>
<td>46.4±26.8</td>
<td>56±30.8</td>
<td>50.65±25.2</td>
<td>44.84±23.8</td>
<td>.005</td>
</tr>
<tr>
<td>Duration of stimulation</td>
<td>8.63±1.9</td>
<td>8.32±2.1</td>
<td>9.78±1.9</td>
<td>8.45±1.9</td>
<td>.001</td>
</tr>
<tr>
<td>Number of follicles&gt;18mm on hCG day</td>
<td>3.22±2.2</td>
<td>2.92±1.8</td>
<td>5.34±2.5</td>
<td>2.67±1.6</td>
<td>.001</td>
</tr>
<tr>
<td>Size of dominant follicle</td>
<td>20.1±1.6</td>
<td>19.95±1.9</td>
<td>20.17±1.4</td>
<td>20.13±1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Endometrial thickness on hCG day</td>
<td>12.46±1.67</td>
<td>8.52±1.6</td>
<td>10.95±1.8</td>
<td>12.28±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Endometrial thickness on puncture day</td>
<td>12.9±1.7</td>
<td>10.19±1.6</td>
<td>12.85±1.8</td>
<td>12.91±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Number of retrieved oocytes</td>
<td>3.52±2.6</td>
<td>3.36±3.2</td>
<td>4.7±3.05</td>
<td>3.26±2.9</td>
<td>.001</td>
</tr>
<tr>
<td>Number of MII oocytes</td>
<td>3.07±2.5</td>
<td>2.67±2.3</td>
<td>3.69±3.1</td>
<td>2.65±2.2</td>
<td>.001</td>
</tr>
<tr>
<td>Number of embryos transferred</td>
<td>1.3±1</td>
<td>1.6±1.1</td>
<td>1.6±1.1</td>
<td>1.3±1.1</td>
<td>NS</td>
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<tr>
<td>Implantation rate (%)</td>
<td>4.9</td>
<td>6.5</td>
<td>8.5</td>
<td>6.6</td>
<td>NS</td>
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<tr>
<td>Chemical pregnancy rate (%)</td>
<td>8</td>
<td>11.3</td>
<td>15.3</td>
<td>13.5</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>8</td>
<td>11.3</td>
<td>15.3</td>
<td>11.5</td>
<td>NS</td>
</tr>
<tr>
<td>Overall cancellation rate (%)</td>
<td>5.9</td>
<td>5.6</td>
<td>3</td>
<td>6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Notes: Data are expressed as mean±SD. NS, not statistically significant

a Short versus miniflare, P=.03
b Long versus antagonist, P=.001 - Long versus short, P<0.001 - Long versus miniflare, P<0.001
c long versus antagonist, P=.026 - Long versus short, P=0.002 - Long versus miniflare, P<0.001
d long versus miniflare, P=.008 - e long versus miniflare, P=.002
References


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a retrospective study

Running Title

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Key words: GnRH agonist; GnRH antagonist; IVF; poor responder
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Data analysis was performed using the Statistical Package for Social Sciences (SPSS) version 16.0. For normally distributed data, analysis of variance (ANOVA) was used to compare the mean values between different stimulation protocols. For data that were not normally distributed (number of follicles>18mm on hCG day), a Kruskal-Wallis test was used to compare the mean values between the stimulation protocols. The chi-square test was used to compare implantation rate, chemical and clinical pregnancy rates and overall cancellation rate among four groups. A P value of <.05 was considered statistically significant.

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According to our findings, the use of ovarian protocols have similar IVF outcomes in terms of implantation rate, chemical and clinical pregnancy rates and overall cancellation rate in poor respond women. Some other studies comparing different stimulation protocols in poor responders similarly showed no significant differences between the clinical results of stimulation regimens.
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Our study has some obvious limitations in terms of its retrospective nature and significant age
between groups. Our data analysis revealed that poor responders under the age of 38 years have
more retrieved and mature oocytes than their peers over 38 years but no relationship between age
and other clinical outcomes such as pregnancy, implantation and cancellation rates were
achieved. Therefore, superior results in terms of retrieved oocytes and mature oocytes in long
protocol group might be due to younger age in this group of patients. However, in a study with
retrospective design, managing the similar patients' characteristics between groups cannot be
guaranteed.

One of the most common problems faced in poor responder studies is no existence of a unique
global accepted definition of the poor responders and a variety of criteria have been used in
studies[10]. In our study, different criteria used for selection of poor responders have led to a
heterogeneous study population. However, the results might differ with the application of more
strict and unique criteria for this group of patients.

Ultimately, the present study demonstrates that the application of four different protocols in poor
respond patients seems to have similar efficacy in improving clinical outcomes such as
implantation and pregnancy rates, eventhough GnRH-a long protocol yielded more retrieved
oocytes and mature oocytes compared to GnRH-a miniflare protocol.

There are insufficient evidences to support the definite intervention in the management of poor
responders undergoing IVF. Thus, further prospective trials in more homogenous population
would be necessary to determine the optimum COH protocol to improve the outcomes of this patient population.

Declaration of interest

The authors report no declarations of interest.
Table I. Patient characteristics

<table>
<thead>
<tr>
<th>characteristics</th>
<th>Antagonist</th>
<th>Short</th>
<th>Long</th>
<th>Miniflare</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cycles</td>
<td>72</td>
<td>73</td>
<td>136</td>
<td>285</td>
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<tr>
<td>Age (years)</td>
<td>37.5±5.15</td>
<td>39.19±4.38</td>
<td>35.58±6</td>
<td>38.06±5.18</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Duration of infertility</td>
<td>10.31±6.7</td>
<td>11.86±6.6</td>
<td>10.59±6.66</td>
<td>10.65±6.97</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index</td>
<td>23.5±9.51</td>
<td>22.8±10.03</td>
<td>24.2±8.65</td>
<td>23.7±9.43</td>
<td>NS</td>
</tr>
</tbody>
</table>

Notes: Data are expressed as mean±SD

NS, not statistically significant
Table II. Cycle characteristics and clinical outcomes

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Number of gonadotropin ampoules</td>
<td>46.4±26.8</td>
<td>56±30.8a</td>
<td>50.65±25.2</td>
<td>44.84±23.8</td>
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<tr>
<td>Duration of stimulation</td>
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<td>9.78±1.9b</td>
<td>8.45±1.9</td>
<td>.001</td>
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<tr>
<td>Number of follicles&gt;18mm on hCG day</td>
<td>3.22±2.2</td>
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<td>Size of dominant follicle</td>
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<td>20.13±1.9</td>
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<td>12.46±1.67</td>
<td>8.52±1.6</td>
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<td>12.9±1.7</td>
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<td>Number of retrieved oocytes</td>
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<td>Number of MII oocytes</td>
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<td>Number of embryos transferred</td>
<td>1.3±1</td>
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<td>Implantation rate (%)</td>
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