Study on Differences in Spermatogenic Suppression between Azoospermic and Oligozoospermic Responders Treated with Levonorgestrel Implant Plus Testosterone Undecanoate Injectable in Chinese Men

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Objective To investigate possible causes resulting in the differences in the spermatogenesis suppression on individual treated with levonorgestrel (LNG) implants and testosterone undecanoate (TU) injectable

Methods Totally 21 Chinese male volunteers were given treatment with LNG implants (four rods, 75 mg/rod) and intramuscular injection of TU (500 mg, bimonthly for 3 times). According to the effects of treatment, they were divided into two groups, namely, azoospermia group (group A) and oligozoospermia group (group O). Then seminal FSH, LH, T and estradiol (E₂) were determined by immunoenzymetric assay, while seminal and serum dihydrotachysterol (DHT) and serum sex hormone binding globulin (SHBG) were by radioimmunoassay, and seminal transferrin (Tf) by scatter turbidimetry assay.

Results Seminal FSH, LH and serum DHT, SHBG, FTI (T/SHBG × 100) levels were significantly lower in group A than in group O, while higher seminal concentrations of E₂ were observed in azoospermia group.

Conclusion The differences in the spermatogenic suppression in Chinese men might be attributed to different rate of peripheral androgen metabolism, variations in serum SHBG levels, 5α-reductase activity and individual aromatase activity during LNG plus TU administration. In addition, seminal sex hormones might be more sensitive indexes to assess the extent of feedback inhibition on hypothalamus-pituitary-testis with exogenous testosterone plus progestogen in the efficacy hormone male contraceptive trials.

Key words: heterogeneity; levonorgestrel (LNG); male contraception; spermatogenesis; testosterone undecanoate(TU)

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Recently there have been a variety of approaches to the development of an effective reversible male contraceptive \[1\]. The most promising method to date is testosterone analogue alone or combined with progestogen, such as testosterone enanthate (TE) 200 mg injection weekly induced to azoospermia in 40%~70% Caucasian \[2\] and testosterone undecanoate (TU) about 90% in Chinese men \[3\]. The remainders are rendered oligozoospermia (sperm density $<20 \times 10^6$/mL), who also have low rate of spermatogenic potential associated with more pregnancies than azoospermia \[4,5\]. While the causes for heterogeneity of spermatogenic suppression remains unclear. There are good evidences in vivo and in vitro to demonstrate that fertility capacity could not be abolished completely even when gonadotropins were suppressed to undetectable levels \[6\] and data in rats also showed that Leydig cells continued to secrete small amount of testosterone (T) after hypophysectomy \[7\]. It is, therefore, important to further investigate the causes or mechanism(s) responsible for the maintenance of residual spermatogenesis during hormonal suppression.

No major differences have been identified in physical or hormonal characteristics or pharmacokinetics and pharmacodynamics between azoospermic and oligozoospermic responders in Caucasian subjects \[8,9\], which was unlikely to account for the heterogeneity in spermatogenic suppression. However, no similar studies have been performed in Chinese men so far. We, thereby, compared the serum and seminal hormones levels and other parameters in 21 Chinese male volunteers treated with levonorgestrel (LNG) implants combined with TU injection so as to identify possible reasons for differences between azoospermic and oligozoospermic responders.

**Materials & Methods**

**Subjects**

Twenty-one healthy fertile Chinese men, aged 25~35, were enrolled in this study. All of them had normal medical history and went through physical examinations and screening laboratory tests. For all the subjects, their serum gonadotropins and T levels were within normal range and their basal sperm counts were $>20 \times 10^6$/mL. The Ethical Review Committee of the Shanghai Institute of Planned Parenthood Research approved the study, and all subjects had signed informed consent documents before participation.

**LNG implants and TU injectable**

Each of the two-rod LNG implant (Shanghai Dahua Pharmaceutical Company, Shanghai, China) contains 150 mg LNG (75 mg/rod). The injectable TU (Xianju Pharmaceutical Company, Zhejiang Province, China) was suspended in tea-seed oil at a concentration of 250 mg/mL. The same batch of TU injectable and LNG implants were used throughout the study.
Study design

The study consisted of 3 periods: a 4-week control, a 24-week treatment and a 12–16-week recovery. At the beginning of treatment period, 4 LNG rods were implanted under the skin of the upper arm of each subject. Four weeks after insertion, each subject began receiving TU injection at a dose of 500 mg at 8 weeks' interval for 24 weeks. At the end of treatment period, the LNG implants were removed. After the treatment period, the subjects were followed until serum T, FSH and LH had recovered, and sperm counts were \(>20 \times 10^6/\text{mL}\) on two consecutive occasions.

Throughout the study, subjects undertook a monthly physical examination and were also asked to fill in a questionnaire about their general health status, libido and sexual function each time. Blood and sperm fluid samples were taken and serum sex hormone binding globulin (SHBG), dehydroepiandrosterone (DHT), as well as seminal FSH, LH, T, E\(_2\), DHT, transferrin (Tf) were measured at weeks -1, 0, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36 in all subjects (the day of LNG implantation was established as baseline day 0). All serum samples were centrifuged and stored at \(-70^\circ\text{C}\) until analysis.

Measurements

Semen analyses were performed according to WHO Laboratory Manual for Examination of Human Semen and Sperm-Cervical Mucus Interaction (3rd edition)\(^{[10]}\). Azoospermia was defined as two or more consecutive sperm counts of zero, while oligozoospermia as two or more counts <20 \(\times 10^6/\text{mL}\).

Seminal FSH, LH, T and E\(_2\) levels were measured by immunoenzymometric assay (Serozyme, Italy). The sensitivity of FSH was 0.3 IU/L, intra- and inter-assay coefficients of variation were 4.6% and 13.8%, respectively. The sensitivity of LH was 0.35 IU/L, intra- and inter-assay coefficients of variation were 5.0% and 14.5%, respectively. The sensitivity of T was 0.4 nmol/L, intra- and inter-assay coefficients of variation were 6.3% and 14.0%, respectively. The sensitivity of E\(_2\) was 0.05 pg/mL, intra- and inter-assay coefficients of variation were 5.7% and 12.4%.

Serum and seminal DHT was measured by radioimmunology assay (Diagnostic Systems Laboratories, USA), serum SHBG was measured by radioimmunoassay assay (Orion, Finland), and seminal Tf was measured by scatter turbidimetry assay (Orion, Finland). The sensitivity of DHT was 2.0 pg/mL, intra- and inter-assay coefficients of variation were 5.0% and 11.5%, respectively. The sensitivity of SHBG was 0.5 nmol/L, intra- and inter-assay coefficients were 4.3% and 8%. The sensitivity of Tf was 0.1 mg/L.

Statistics

All variables were checked for normal distribution in the one sample Kolmogorov-Smirnov test for goodness of fit. Variations between study groups were evaluated by two-way ANOVA with Neuman-Keuls test for post-hoc analysis for repeated measurements. In the case of a single missing value per time point, the appropriate mean was inserted to allow ANOVA for
repeated measurements. When necessary, those data that did not fit normal distribution were logarithmically transformed for analysis. All data were \( \bar{x} \pm s_x \). For all analyses a two-side \( P \) values of 0.05 was considered significant. Analyses were performed by SPSS 10.0.

**Results**

Subjects were divided into azoospermia group (group A, 13 subjects) and oligozoospermia group (group O, 8 subjects) during week 14~28 of the treatment period. There were no significant differences in height, weight, body mass index (BMI), body surface area (BSA), and testis volume between subjects in the two groups (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>BSA (m²)</th>
<th>Testis volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>13</td>
<td>31.67±0.56</td>
<td>165±1.93</td>
<td>65.10±1.26</td>
<td>22.13±0.36</td>
<td>1.85±0.15</td>
<td>20.21±2.8</td>
</tr>
<tr>
<td>Group O</td>
<td>8</td>
<td>32.76±0.50</td>
<td>170±1.27</td>
<td>70.85±3.40</td>
<td>24.53±0.93</td>
<td>1.87±0.17</td>
<td>21.32±3.2</td>
</tr>
</tbody>
</table>

BMI = body mass index  
BSA = body surface area

**Sperm density**

There were no pretreatment differences in sperm density \((86.0±20.6 \times 10^6/mL vs 84.2±11.2 \times 10^6/mL)\) between group A and O \((P>0.05)\) before treatment. In group A, all subjects had developed azoospermia from 8~28 weeks after implantation. In group O, the mean time to decline to the lowest sperm density \((1.9~2.6\times 10^6/mL)\) was 19.5±1.05 weeks. There were no significant differences in the decreasing rate of sperm production between the two groups \((P>0.05)\) and sperm densities in all subjects recovered \( (>20\times 10^6/mL) \) within 10 weeks after the removal of implant devices.

As there were no significant differences in serum FSH, LH, T and LNG levels between the two groups throughout the study, we further determined the serum SHBG, DHT, FTI \((FTI=T/SHBG \times 100)\) \[^{11}\] levels and seminal T, FSH, LH, E\(_2\), DHT, Tf levels to explore potential mechanism for the marked individual differences in spermatogenesis suppression.

**Seminal parameters**

There were no pretreatment differences in seminal FSH, LH, T, Tf, DHT, and serum DHT, SHBG levels and FTI between the two groups except that seminal E\(_2\) baseline levels were significantly higher in group A than those in group O (Table 2).

There was no difference in seminal T, DHT and Tf levels between the two groups. Suppression of seminal FSH and LH levels was more significant in subjects with azoospermia than those with oligozoospermia after implantation. However, they reached statistical differences at only one time point (FSH, at week 16, \( P<0.005 \); LH, at week 28, \( P<0.004 \)), respectively. Seminal E\(_2\) concentrations were higher significantly in group A than those in
group O at week 6, 8, 12, 24 (P<0.0001) (Table 2).

Table 2 Seminal parameters at baseline (weeks -1,0), during treatment (weeks 2,4,8,12,16,20,24,28) and recovery (weeks 32, 36) (x ± s.)

<table>
<thead>
<tr>
<th>Period</th>
<th>Week</th>
<th>FSH (IU/L)</th>
<th>LH (IU/L)</th>
<th>T (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group A</td>
<td>Group O</td>
<td>Group A</td>
</tr>
<tr>
<td>Control baseline</td>
<td>-1</td>
<td>1.00±0.15</td>
<td>1.47±0.42</td>
<td>1.26±0.48</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.11±0.19</td>
<td>1.29±0.45</td>
<td>0.88±0.12</td>
</tr>
<tr>
<td>Treatment</td>
<td>2</td>
<td>0.82±0.16</td>
<td>0.94±0.33</td>
<td>1.04±0.14</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.75±0.17</td>
<td>1.04±0.36</td>
<td>0.70±0.11</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.62±1.10</td>
<td>0.83±0.29</td>
<td>0.48±0.07</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.81±0.25</td>
<td>0.80±0.29</td>
<td>0.34±0.06</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0.46±0.08</td>
<td>1.03±0.37</td>
<td>0.38±0.10</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.67±0.16</td>
<td>0.84±0.30</td>
<td>0.92±0.54</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.57±0.11</td>
<td>0.93±0.33</td>
<td>0.32±0.04</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>0.71±0.17</td>
<td>0.89±0.31</td>
<td>0.40±0.12</td>
</tr>
<tr>
<td>Recovery</td>
<td>32</td>
<td>0.33±0.24</td>
<td>1.02±0.36</td>
<td>0.92±0.19</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>1.10±0.19</td>
<td>1.31±0.46</td>
<td>0.58±0.11</td>
</tr>
</tbody>
</table>

*: significant changes described in text

Serum parameters

Serum DHT and SHBG levels reduced in all subjects after implantation and remained at similar levels during TU injections and then recovered after removal of implants. Suppression of serum DHT (weeks 4,12: P<0.0001) and SHBG levels (weeks 12, 16, 24, 28; P<0.0001) was more significant different in group A compared with that in group O. Despite that serum DHT and SHBG concentrations appeared higher in group O during recovery, no significant differences were found between group A and O (P>0.05). Values of serum FTI elevated slightly in group A, while reduced significantly in group O after implantation. Serum FTI in all subjects recovered in both groups after removal of implants. Values of serum FTI were significantly higher in group A than group O at weeks 4, 16, 28 (P<0.0001) (Table 3) after implantation.
Table 3  Serum parameters at baseline (weeks -1,0), during treatment (weeks 2,4,8,12,16,20,24,28) and recovery (weeks 32, 36) ($\bar{x} \pm s$)

<table>
<thead>
<tr>
<th>Period</th>
<th>Week</th>
<th>DHT(pg/mL)</th>
<th>SHBG(nmol/L)</th>
<th>FTI(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group A</td>
<td>Group O</td>
<td>Group A</td>
</tr>
<tr>
<td>Control baseline</td>
<td>-1</td>
<td>224.5±22.1</td>
<td>268.6±6.99</td>
<td>43.7±4.75</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>262.0±26.0</td>
<td>243.1±6.61</td>
<td>44.7±6.38</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>142.3±8.94</td>
<td>182.9±7.52</td>
<td>29.7±5.12</td>
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<tr>
<td></td>
<td>4</td>
<td>121.3±8.99</td>
<td>221.7±5.55</td>
<td>32.2±5.81</td>
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<tr>
<td></td>
<td>8</td>
<td>176.3±13.3</td>
<td>186.1±5.78</td>
<td>28.5±2.89</td>
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<tr>
<td></td>
<td>12</td>
<td>177.4±14.8</td>
<td>197.2±5.40</td>
<td>24.8±3.49</td>
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<tr>
<td></td>
<td>16</td>
<td>159.0±15.5</td>
<td>164.6±6.70</td>
<td>26.5±3.83</td>
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<tr>
<td></td>
<td>20</td>
<td>135.9±8.07</td>
<td>164.9±6.69</td>
<td>24.1±3.24</td>
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<tr>
<td></td>
<td>24</td>
<td>199.2±13.9</td>
<td>172.3±8.39</td>
<td>35.1±3.59</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>215.2±13.9</td>
<td>264.9±8.90</td>
<td>31.4±6.34</td>
</tr>
<tr>
<td>Recovery</td>
<td>32</td>
<td>215.2±15.3</td>
<td>305.7±6.88</td>
<td>55.2±9.41</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>273.7±24.8</td>
<td>354.0±8.29</td>
<td>52.9±4.90</td>
</tr>
</tbody>
</table>

*: significant changes described in text

Serum T/DHT reduced significantly in all subjects after implantation and there were no pretreatment differences in values of serum T/DHT between group A and O ($P>0.05$). Values of T/DHT ratio were higher significantly in group A at weeks 4, 12, 28, 32 than those in group O ($P<0.005$) (Figure 1).

![Figure 1 Mean values of serum T/DHT ratio ($\bar{x} \pm s$) in azoospermic and oligozoospermic responders during LNG implants plus TU injectable](image-url)
Discussion

Results showed that there were no significant differences between the two groups either in the decrease rate of sperm production or in the rate of recovery for sperm production during treatment period. This suggests that the rates of decrease and recovery of sperm production could not explain the causes for the heterogeneity in spermatogenic suppression in Chinese men. This result was different from findings of Wallace EM, et al., that faster rates of decrease and recovery of sperm production were found in Caucasian subjects who had azoospermia. The discrepancy might due to ethnic differences and/or different regimens used in different studies; furthermore, this also suggested that the rate of spermatogenic suppression may not solely depend on hormones, but also associated with intrinsic variations in spermatogenic kinetics.

Seminal FSH and LH concentrations were higher significantly in group O than those in group A (\(P<0.05\)), while no differences occurred in serum gonadotropins, indicating that seminal gonadotropins levels may be more sensitive indexes to assess the extent of spermatogenic suppression resulting from inhibition of gonadotropin secretion. However, except for only one time point showing statistical difference, no consistent differences occurred between the two groups during treatment phase, which proved that seminal gonadotropin level is also unlikely to be exclusively responsible for partial maintenance of spermatogenesis in some subjects.

Higher serum and seminal T levels were found in group A, while there was no statistical difference between the two groups. Results showed that serum SHBG levels were significantly lower in group A, which was similar to the results of the study with testosterone bucillate (TB) injection for male contraception. In addition, after implantation, there were a tendency of significantly lower serum FSH and higher serum DHT levels in group O, which were in accord with the results of previous studies on testosterone enanthate (TE) and TB injection respectively, thus indicating that 5α-reductase activity might increase in group O during treatment. A clinical study also demonstrated that an increase in 5α-reductase activity was observed in men remaining oligozoospermic after exogenous testosterone administration to group A of Caucasian men, but not in those developed azoospermia.

It has been hypothesized that Chinese men might have less 5α-reductase activity as compared with Caucasian men as indicated in some studies. A later study, however, demonstrated that no significant differences in 5α-reductase activity were detected between Chinese and Caucasian men, as well as type I and II of 5α-reductase in foreskin, which showed that 5α-reductase activity alone still seemed unlikely to account for heterogeneity of suppression of spermatogenesis. However, there were much large variable ranges of two isoenzymes of 5α-reductase, type I and II in vivo in normal adult men between and within different population groups. What's more, subjects in that trial did not take any
contraceptives throughout the study so its data could not show whether the activity of 5α-reductase increased or not during treatment. In the present study, values of serum T/DHT ratio were significantly higher in group A than those in group O after implantation. Our findings indicated there was a significant increase in 5α-reductase activity during treatment in group O, while the reason was unclear. It has been proposed that, 5α-reductase activity in the mature adult testis is low when the concentration of T produced by the Leydig cells are high enough to maintain spermatogenesis. When gonadotropins secretion was suppressed to undetectable levels and endogenous testosterone reduced greatly, 5α-reductase androgens may become disproportionately important in the maintenance of spermatogenesis in group O, in whom higher activity has been demonstrated. Up to now, however, direct evidence for this hypothesis is still unavailable. T is peripherally converted to E2 by aromatase. Several studies demonstrated that no significant differences in serum E2 levels were detected between the two groups during all phases of the study. The present results showed that seminal E2 levels in group A was significantly higher at several time points (including -1 week in control period) than those in group O. It is the difference in seminal E2 levels among population or higher serum free T levels and aromatase activity in some individuals who had azoospermia that caused a significant increase in the conversion of free T to E2. The significance of higher serum E2 concentrations during T treatment is unclear as was found in oligozoospermic subjects in another study on TE. Our results indicated that activity of aromatase in seminal fluid might be one of the main reasons for individual differences of spermatogenic suppression. Furthermore, it was supposed that the seminal E2 levels might be a baseline marker for screening those who can utilize this contraceptive or not.

No significant differences in seminal DHT levels were detected between the two groups, which suggested that seminal DHT concentrations were possibly not responsible for the heterogeneity of spermatogenic suppression. No similar reports have been found so far.

It has been demonstrated that transferrin, as a specific marker to assess the function of Sertoli cells and spermatogenic status of seminiferous tubule, is more sensitive than other parameters, such as FSH, T and so on. The results showed that concentrations of seminal T if reduced after implantation, the function of Sertoli cells and seminiferous tubule were also suppressed in subjects treated by LNG implants plus TU injections. However, no significant differences were detected in seminal T if at any time point during the whole course between the two groups, which indicated that the function of Sertoli cells and spermatogenic status of seminiferous tubule could not explain the marked individual differences in suppression of spermatogenesis in Chinese men. Zalata A. et al. had found similar results in the role of androgens in seminal plasma.

To sum up, our results showed that differences in the degree of spermatogenic suppression might be attributed to the different rate of peripheral androgen metabolism, variations in SHBG levels and 5α-reductase activity during treatment, individual aromatase activity in
Chinese men using exogenous androgen plus progestogen. In addition, seminal sex hormones might be more sensitive indexes to assess the extent of feedback inhibition on hypothalamus-pituitary-testis in the trial of efficacy of male hormonal contraceptives.

References


(Received on December 22, 2003)

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ACKNOWLEDGMENT

The Editorial Board of the Journal of Reproduction and Contraception extends its gratitude to the following experts for the unique contribution to the Journal in reviewing the papers in the year of 2003.

Dr Sulochana Gunasheela
Gunasheela Surgical and Maternity Hospital,
Bangalore, India

Dr Hsiu-mei Hsieh-Li
Medical Science Research Institute
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