The Relationship between Serum Hormone Levels and Reproductive Ability in Aging Male Rats

Kazuyoshi HASHIZUME**, Yasushi IKARASHI***, Sadashige SAKUMA and Yutaka SAKAI*

Pathology and Toxicology Function, Japan Upjohn Research Laboratories, 168 Ohyagi, Takasaki, Gunma 370, Japan, and *Department of Pharmacology, National Defense Medical College, Tokorozawa, Saitama 359.

(Received 8 June 1983 / Accepted 7 December 1983)

The relation between fertility and hormonal levels was studied in aging male rats. Reproductive ability was inspected at 30, 31, 59, 60, 93 and 94 weeks of age, and the serum hormone levels were determined by the radioimmunoassay. Reproductive ability decreased with aging (mating rates: 15/20, 11/20, 3/20 and 6/20 at 30, 31, 59 and 60 weeks, respectively) and no male mated at 93 and 94 weeks. Serum testosterone levels in males without reproductive activity were significantly lower than those in males with reproductive activity. LH and FSH showed the lowest level in 95 weeks. The highest value of LH was found at 60 weeks in males without reproductive activity having low testosterone levels. The highest value of prolactin appeared in 95 weeks which was three times higher than those in the other groups. The results suggest that the loss in the reproductive activity in aging rats starts with a decrease in libido, which is followed by a high gonadotropin and low testosterone condition, and accomplished under a low gonadotropin and high prolactin circumstance.

Many mammals show a gradual decline of reproductive function with aging[1,24]. This progressive reduction is a part of the process of senescence and it certainly depends on the age-related changes of the hypothalamus, pituitary gland and gonads functions.

There are many evidences showing a decrease of male reproductive ability with aging in rats; the decline in fertility, decreases in serum testosterone, reduction of testicular steroidogenesis, lowering of gonadotropin level and elevating of prolactin level[3,5,7,17]. However, there is no satisfactory explanation for the relationship between fertility and these changes.

The present study was undertaken in an attempt to examine whether hormone levels [LH, FSH, prolactin (PRL) and testosterone] reflect the decrease of reproductive function in aging male rats.

Materials and Methods

Animals: Wistar-Imamichi rats, 4 weeks of age, were purchased from Imamichi Institute for Animal Reproduction(Ohmiya, Saitama, Japan). The rats were kept in a controlled environment (temperature, 22 ± 2°C; relative humidity, 55 ± 5%; light/dark cycle, 12 hours/12 hours). The rats were given chow diet (CMF, Oriental Yeast

Present address: **Center of Laboratory Animal Science, National Defense Medical College, Tokorozawa, Saitama 359, Japan (防衛医科大学校). ***Research Laboratories for Applied Toxicology, Nippon Kayaku Co., 239 Iwahana, Takasaki, Gunma 370-12, Japan (日本化薬株式会社安全性研究所).
Co., Japan) and water ad libitum.

All males were housed in two per cage throughout the experimental period except during the period of the fertility test and were confirmed to have reproductive ability at 12 weeks of age. At 30, 59 or 93 weeks of age, each different 20 males were used for the experiment. Blood samples were collected from the abdominal aorta under ether anesthesia between 10:00-12:00 hr after the fertility test. Sera were stored at −20°C until the hormones were assayed. Adrenal glands, pituitary gland, testes, prostate glands and epididymides were weighed and epididymides were fixed in a 10% buffered formalin solution for pathological examinations.

Fertility test: Each 20 males were tested at 30, 59 and 93 weeks of age. They were individually placed with a proestrus female which had shown at least 3 consecutive 4-day estrous cycle before the test. The second fertility test was 1 week later at 31, 60 or 94 weeks using another female. The criterion for a male with reproductive ability is the occurrence of pregnancy in a paired female by either of the two tests. Pregnant females were sacrificed 18-22 days after mating.

Hormone assay: Serum LH, FSH and PRL were determined by respective double antibody radioimmunoassays using NIAMDD kits. Serum hormones were assayed in duplicate, and values were expressed as NIAMDD-Rat-PR-1 ng/ml serum. Testosterone was assayed in duplicate by RIA using RIA kit (Eiken, Tokyo) after sera were extracted with ether-hexane. The least detectable testosterone value is 0.24 ng/ml serum.

Statistical analysis: Statistical analysis was made using the Student’s t or the χ² test.

Results

Fertility test: The frequency of mating and the number of male rats with reproductive activity declined with aging as shown in Table 1.

In 30 weeks group, 15 of 20 males mated at the 1st test and 11 of 20 at the 2nd test. Thirteen of 15 and 11 of 11 mated females had live fetuses. The number of males mated at the first and/or the second tests was 17 of 20 and 15 of them impregnated. At 59-60 weeks, only 3 and 6 males mated, respectively. Seven of 20 males mated at the 1st and/or the 2nd tests, 5 of which were fertile. Although all 20 males in 95 weeks group contained a considerable amount of spermatozoa in the epididymis, none of them mated.

Organ weights: Organ weights relating to reproduction are shown in Table 2.

Body weight increased during the period of 31 to 60 weeks (P < 0.01). The average body weight in the group of males without reproductive activity at 60 weeks was significantly heavier than that in the

<table>
<thead>
<tr>
<th>Table 1. Breeding capability in male rats with aging</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (Weeks)</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>31</td>
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<tr>
<td>59</td>
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<td>60</td>
</tr>
<tr>
<td>93</td>
</tr>
<tr>
<td>94</td>
</tr>
</tbody>
</table>

Mean ± S. D.

*Total number of males mated at the 1st and/or the second tests.
Table 2. Organ weights in rats with aging

<table>
<thead>
<tr>
<th>Group (Age, weeks)</th>
<th>Reproductive activity</th>
<th>No. of rat</th>
<th>Body weight (g)</th>
<th>Adrenal (mg)</th>
<th>Pituitary (mg)</th>
<th>Testes (g)</th>
<th>Prostate (g)</th>
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</thead>
<tbody>
<tr>
<td>31</td>
<td>+</td>
<td>17</td>
<td>653.7±77.1</td>
<td>67.0±13.6</td>
<td>12.6±3.7</td>
<td>3.18±0.20</td>
<td>0.71±0.15</td>
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<tr>
<td></td>
<td>−</td>
<td>3</td>
<td>678.3±40.5</td>
<td>68.0±16.7</td>
<td>14.4±5.4</td>
<td>3.04±0.04</td>
<td>0.74±0.16</td>
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<tr>
<td>60</td>
<td>+</td>
<td>5</td>
<td>675.2±26.8</td>
<td>66.1±13.6</td>
<td>11.1±4.5</td>
<td>3.21±0.07</td>
<td>0.83±0.11</td>
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<tr>
<td></td>
<td>−</td>
<td>15</td>
<td>772.6±76.2**</td>
<td>66.6±10.8</td>
<td>13.6±1.5</td>
<td>3.18±0.14</td>
<td>0.90±0.20**</td>
</tr>
<tr>
<td>95</td>
<td>+</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>20</td>
<td>720.8±85.5*</td>
<td>44.3±13.2**</td>
<td>15.7±4.7**</td>
<td>3.06±0.31</td>
<td>0.94±0.28**</td>
</tr>
</tbody>
</table>

Mean ± S. D.
*P<0.05. **P<0.01.
Significantly different from 31 weeks group of males with reproductive activity.

Table 3. Serum hormone levels in rats with aging

<table>
<thead>
<tr>
<th>Group (Age, weeks)</th>
<th>Reproductive activity</th>
<th>No. of rat</th>
<th>FSH (ng/ml)</th>
<th>LH (ng/ml)</th>
<th>PRL (ng/ml)</th>
<th>Testosterone (ng/ml)</th>
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<tbody>
<tr>
<td>31</td>
<td>+</td>
<td>17</td>
<td>277.5±10.7</td>
<td>24.7±3.4</td>
<td>18.3±1.7</td>
<td>3.44±0.28</td>
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<td>−</td>
<td>3</td>
<td>308.6±39.3</td>
<td>29.7±9.7</td>
<td>19.9±6.0</td>
<td>3.50±0.43</td>
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<tr>
<td>60</td>
<td>+</td>
<td>5</td>
<td>268.2±20.3</td>
<td>22.6±3.7</td>
<td>19.9±3.1</td>
<td>3.00±0.43</td>
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<tr>
<td></td>
<td>−</td>
<td>15</td>
<td>255.0±10.5</td>
<td>39.9±2.8**</td>
<td>18.1±0.9</td>
<td>2.60±0.24*</td>
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<tr>
<td>95</td>
<td>+</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>20</td>
<td>192.0±12.2**</td>
<td>12.8±2.9*</td>
<td>54.3±14.9*</td>
<td>2.49±0.31*</td>
</tr>
</tbody>
</table>

Mean ± S. E. (ng/ml).
*P<0.05. **P<0.01.
Significantly different from 31 weeks group of males with reproductive activity.

Discussion

The results of this study showed that the decrease in the reproductive ability with aging had some relation with hormone levels (LH, FSH, PRL and testosterone).

Males at 60 weeks of age had lower mating rate and reproductive activity than those in previous reports [7, 10, 23]. The discrepancy may possibly be arisen from less opportunities for mating before the reproduction test in this study (twice at
12 weeks). The repetition of copulatory opportunities or mating is believed to stimulate reproductive ability and maintain fertility [25].

Copulatory ability and mating behavior were defective in 95 weeks old rats. However, the males without reproductive activity had many spermatozoa in the epididymis and testis. Takeshima et al. [23] stated that spermatozoa in males without reproductive activity over 90 weeks in the Wistar Imamichi strain had fertility. From these facts, the decline of reproductive ability in this study may be attributed to the lack of copulatory response or mounting behavior.

Hyperprolactinemia suppresses the copulatory behavior response [6, 8, 13, 16, 22]. The males without reproductive activity at 60 weeks in this study did not reveal higher levels of PRL but males at 95 weeks showed the highest value of PRL. PRL increased with aging and hyperprolactinemia may be concerned with the infertility in males. In addition, it is possible to speculate that male infertility might not be caused by hyperprolactinemia, but by the process of senescence changes such as dysfunction of pituitary, pituitary tumor and other endocrine disorder in the aging rats.

A lower testosterone level was found in males without reproductive activity and it decreased with aging. This result indicates that non-copulatory males have lower testosterone level than normal ones. The decrease in serum testosterone level with aging is consistent with that in other reports [7, 9, 11, 14], whereas many investigators stated that there was no relationship between the testosterone level and sexual activity [3, 5, 15]. It seems that the decline in sexual activity is not caused by lower testosterone level but the lower testosterone level reflects the decline in reproductive activity.

LH, FSH and testosterone levels were the lowest in 95 weeks old males. The LH level at 60 weeks in the group of males without reproductive activity was significantly higher than that in any other groups. The hypothalamus and pituitary axis seemed to be still functional at 60 weeks in the group of males without reproductive activity, because both the testosterone and LH level changed simultaneously [9, 12, 18, 19, 20]. At 60 weeks the LH and testosterone levels were markedly different between two groups (group of males with and without reproductive activity). The occurrence of these changes may be followed by hyperprolactinemia and lower gonadotropins. The nature and quality of gonadotropins may have changed with aging and contributed to male infertility [2, 4, 21]. Consequently, further examinations, for example, the changes in the nature of gonadotropins, the pituitary function and their controlling mechanisms, are necessary to elucidate the initial cause for the decline in sexual activity.

Acknowledgements

The authors are indebted to Drs. Y. Hasegawa and M. Igarashi of Department of Obstetrics and Gynecology, Gunma University, for helping of gonadotropins assay.

References


加齢ラットの繁殖能力と血清中ホルモンレベルとの関係

橋爪 一善・五十嵐 康・佐久間貞重・酒井 豊*

日本アップジョン株式会社総合研究所病理毒性部

*防衛医科大学校

雄ラットの加齢による繁殖能の減退と血中ホルモン含量の変化について検討した。繁殖能の無有は生後30.59.93週齢より2週間おわり、それぞれ20匹の雄を用いて交尾および妊娠の成否により判定した。繁殖試験終了後の雄は生後31.60.95週目に採血し剖検した。各時期の血中ホルモンを測定し、繁殖能の有無と対比検討した。生後30.31週齢では85％の繁殖能が認められたが、59.60週齢では35％に減退し、93.94週齢では繁殖能は認められなかった。血中テストステロン量は加齢に伴い低下し、ときに、繁殖能の認められなかった個体ではその低下が著しかった。血中性腺刺激ホルモンのうち、FSH、LH量は加齢とともに低下し95週齢で最低値を示した。一方、プロラクチン量は95週齢で著しい高値を示した。雄の加齢による繁殖能の減退は交尾能の低下に始まり、その後、繁殖能は血中テストステロン量の減少およびプロラクチン量の上昇と深い関連性のあらうことが推察された。