The Effects of Administration of Semen Cuscutae on Ovulation and Developmental Competence in Mice.

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Introduction

Women’s fecundity reaches its highest point at the age of 24, showing a sharp decrease after 35. This decrease of fecundity is due to not so much the reduction of the uterine function as to factors related to the ovary and ova.

Among other ovary-related factors, the smaller number of ova resulting from aging has so far been recognized as a major cause for this decrease of fecundity. According to recent studies, however, qualitative and quantitative changes in ovulation resulting from the decrease of ovarian function due to disorders of blood circulation with age are largely responsible for the decrease of fecundity in fertile women in their mid thirties or older\(^1\).\(^2\).

Semen Cuscutae, a representative medicinal stuff for tonifying Yang, acts on the Kidney meridian (揄樞), fostering original vital function, reinforcing ki and menstrual control. It is also known from pharmaceutical experiments to act on the cardiovascular system and decrease the blood
press). Considering these effects of *Semen Cuscutae*, it is thought that it can be applied to the treatment of the decreased ovarian function in women but no experiment has been done regarding this application yet.

This study was therefore conducted to investigate the effects of *Semen Cuscutae* on ovulation and *in vitro* development competence of 2-cell embryos to blastocyst stage in mice.

**Experiment**

1. Test animals & Medicinal stuff

1) Test animals

Two groups of ICR (Institute of Cancer Research) mice were used for this experiment: one younger group of 4-week-old mice at their highest fecundity and the other an aged group of 18-week-old mice showing significantly lower fecundity. These mice were kept in breeding rooms with a temperature of 21°C ± 2°C, a humidity of 50%, and alternate periods of light and darkness of 12 hours each, and provided with enough water and food.

2) Test medicine material

*Semen Cuscutae* bought in the market was used as test medicine material.

2. Methods

1) Concoction of medicine

30g of *Semen Cuscutae* was boiled in a medical double boiler with 1,200ml of distilled water for 2 hours and the upper part of the solution (250ml) was collected. After being passed through sterilized gauze, the solution was refrigerated at 4°C between uses.

2) Treatment group and control group

Regarding the ovulation and the *in vitro* developmental competence of the fertilized eggs, the medicine was orally administered by a sonde, 0.3ml per mouse, once a day for 4 days (duration of a menstrual period4)) and 8 days to the treatment group of 4-week-old and 18-week-old mice. Plain water was administered to a control group of 4-week-old and 18-week-old mice with the same method as the treatment group.

3) Comparison of fecundity of 4-week-old and 18-week-old mice after administration of the medicine 5 IU of PMSG (Pregnant Mare’s Serum Gonadotropin, Sigma, USA) and HCG (Human Chorionic Gonadotropin, Sigma, USA) was administered to both groups by intraperitoneal injection at intervals of 48 hours. Then, 18 hours after the administration of HCG, some of the mice were killed using cervical dislocation, for the purpose of the extraction of the ovaries. The ovaries were weighed and the oocyte-cumulus cell complexes (OCCs) were collected. OCCs were deprived of oocytes after being dipped in P-1 culture fluid (Irvine Scientific, USA) containing 0.1% hyaluronidase (Gibco, USA). Then, the treatment and control groups were compared in the number of total released ova and normally-shaped ova.

In the meanwhile, after HCG administration, the rest of the mice were each made to mate with a male mouse whose fecundity had been previously verified. The next day the mice who showed signs of fertilization in the vaginal plug were killed 36 hours after HCG administration. The fertilized eggs were collected from the oviducts which had been flushed in P-1 culture fluid. After the number of the fertilized eggs was counted, with a view to examining their *in vitro* developmental competence, they were bred for 96 hours in a culture medium (Hera Cell, Germany) with 5% of CO2 and the temperature of 37°C, which contained...
P-1 culture fluid containing 0.3% BSA (Bovine serum albumin, Sigma, USA), and compared in the developmental rate into blastocyst stage, a step before implantation.

4) Analysis of results & statistical analysis
This experiment was repeatedly conducted at least 5 times and the results were analyzed using the independent t-test. Differences at p<0.05 were considered statistically significant.

**Results**

1. Comparison of fecundity in 4-week-old mice

1) The body, ovarian weight and the ovulation
In the comparison of the body and ovarian weight after *Semen Cuscutae* administration, there was no significant difference between the treatment and control groups.

In the number of total released ova after *Semen Cuscutae* administration for 4 days, that of the treatment group showed significant increase (p<0.05), compared with that of the control group (treatment group 21.4, control group 15.0). For 8 days, that of the treatment group showed significant increase (p<0.001), compared with that of the control group (treatment group 24.5, control group 17.1).

In the comparison of normal ova rate after administration, there was no significant difference between the treatment and control groups.

2) The in vitro developmental competence
In the comparison of the developmental competence from pronuclear stage oocytes into blastocyst stage embryos after *Semen Cuscutae* administration for 4 days, the treatment group showed significant increase (p<0.001), compared with the control group (treatment group 51.7%, control group 21.0%). For 8 days, the treatment group showed significant increase (p<0.01), compared with the control group (treatment group 54.9%, control group 34.5%).

**Table 1. The Effects of Administration of *Semen Cuscutae* on the in vitro Development Competence in 4-week-old Mice**

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Mice</th>
<th>Mean Body Weight (g)</th>
<th>Mean Ovary Weight (g)</th>
<th>No.(mean) of Oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ovulated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Normal Oocyte(%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Abnormal Oocyte(%)</td>
</tr>
<tr>
<td>4-Day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>7</td>
<td>22.0</td>
<td>0.0114</td>
<td>105 (10.5)</td>
</tr>
<tr>
<td>Treatment</td>
<td>12</td>
<td>21.2</td>
<td>0.0112</td>
<td>257 (21.4)*</td>
</tr>
<tr>
<td>8-Day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 3</td>
<td>10</td>
<td>26.2</td>
<td>0.0151</td>
<td>171 (17.1)</td>
</tr>
<tr>
<td>Treatment</td>
<td>10</td>
<td>25.8</td>
<td>0.0161</td>
<td>245 (24.5)**</td>
</tr>
</tbody>
</table>

*: statistical significance between control group & treatment group (p<0.05)
**: statistical significance between control group & treatment group (p<0.001)

1. 4-week-old mice administered plain water as control for 4 days
2. 4-week-old mice administered *Semen Cuscutae* for 4 days
3. 4-week-old mice administered plain water as control for 8 days
4. 4-week-old mice administered *Semen Cuscutae* for 8 days
2. Comparison of fecundity in 18-week-old mice

1) The body, ovarian weight and the ovulation

In the comparison of the body and ovarian weight after Semen Cuscutae administration, there was no significant difference between the treatment and control groups.

In the number of total released ova after Semen Cuscutae administration for 4 days, that of the treatment group showed significant increase (p<0.001), compared with that of the control group (treatment group 30.8, control group 13.3). For 8 days, that of the treatment group showed significant increase (p<0.01), compared with that of the control group (treatment group 29.5, control group 18.6).

In the comparison of normal ova rate after administration, there was no significant difference
between the treatment and control groups.

2) The in vitro developmental competence

In the comparison of the developmental competence from pronuclear stage oocytes into blastocyst stage embryos after Semen Cuscutae administration for 4 days, the treatment group showed significant increase (p<0.001), compared with the control group (treatment group 59.5%, control group 31.0%). For 8 days, the treatment group showed significant increase (p<0.00001), compared with the control group (treatment group 64.2%, control group 16.3%).

Human growth and fecundity depend on the function of the ki of the kidneys (☭ekyll). Somun· Sanggochunjinron (☭ekyll · ☭ekyll · ☭ekyll) says that the ki of the kidneys (☭ekyll), Conception Vessel (☭ekyll) and Penetration Vessel (☭ekyll) have much influence on menstruation and pregnancy and Nanjing· Thirty ninth (☭ekyll · ☭ekyll · ☭ekyll) mentions the relationship between the Vital Gate (☭ekyll) and Uterus. Because the Fire of the Vital Gate (☭ekyll) , sometimes referred to as the Yang of the kidneys (☭ekyll), is an engine to further human growth and breeding and to help reproduction, when it wanes, it can give rise to a decrease of fecundity, finally leading to infertility ☭ekyll.

Semen Cuscutae, a representative medicinal

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Mice</th>
<th>No. (mean) of Embryos Recovered</th>
<th>No. (mean) of Embryos Cultured (%)</th>
<th>In vitro Development to (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4-cell</td>
<td>Morula</td>
<td>Blastocyst</td>
</tr>
<tr>
<td>Control 1</td>
<td>5</td>
<td>104 (20.8)</td>
<td>72 (16.8)</td>
<td>26</td>
</tr>
<tr>
<td>4-Day</td>
<td>Treatment 2</td>
<td>10 278 (27.8)</td>
<td>232 (23.2)</td>
<td>207 (89.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>171 (73.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>138 (59.5%)*</td>
</tr>
<tr>
<td>Control 3</td>
<td>9</td>
<td>201 (22.3)</td>
<td>166 (18.4)</td>
<td>107 (64.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>61 (36.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>27</td>
</tr>
<tr>
<td>8-Day</td>
<td>Treatment 4</td>
<td>10 315 (31.5)</td>
<td>260 (26.0)</td>
<td>244 (93.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>218 (83.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>167 (64.2%)**</td>
</tr>
</tbody>
</table>

*: statistical significance between control group & treatment group (p<0.001)
**: statistical significance between control group & treatment group (p<0.00001)
1. 18-week-old mice administered plain water as control for 4 days
2. 18-week-old mice administered Semen Cuscutae for 4 days
3. 18-week-old mice administered plain water as control for 8 days
4. 18-week-old mice administered Semen Cuscutae for 8 days

Discussion

Women’s fertility reaches its highest point at the age of 24, showing a sharp decrease after 35. This decrease of fecundity is due to not so much the reduction of the uterine function as to factors related to the ovary and ova.

Among other ovary-related factors, the smaller number of ova resulting from aging has so far been recognized as a major cause for this decrease of fecundity. According to recent studies, however, qualitative and quantitative changes in ovulation resulting from the decrease of ovarian function due to disorders of blood circulation with age are largely responsible for the decrease of fecundity in fertile women in their mid thirties or older2.5.

Table 4. The Effects of Administration of Semen Cuscutae on the in vitro Development Competence in 18-week-old Mice
stuff for tonifying Yang, acts on the Kidney meridian (ְַַָ), fostering original vital function, reinforcing ki and menstrual control. It is also known from pharmaceutical experiments to act on the cardiovascular system and decrease the blood press³).

Considering these effects of Semen Cuscutae, it is thought that it can be applied to the treatment of the decreased ovarian function in women, but no experiment has been done regarding this application yet.

Therefore, this experiment was conducted for the purpose of examining the effects of Semen Cuscutae on fecundity in mice. Semen Cuscutae was administered in the form of a decoction and its effects on ovarian functions such as ovulation and the developmental competence from pronuclear stage oocytes into blastocyst stage embryos were observed.

In the comparison of the body and ovarian weight after Semen Cuscutae administration, there was no significant difference between the 4-week-old and 18-week-old (treated) groups and the 4-week-old and 18-week-old (untreated) groups.

In the number of total released ova after Semen Cuscutae administration, that of the 4-week-old and 18-week-old (treated) groups showed significant increase, compared with that of the 4-week-old and 18-week-old (untreated) groups.

In the comparison of normal ova rate after administration, there was no significant difference between the 4-week-old and 18-week-old (treated) groups and the 4-week-old and 18-week-old (untreated) groups.

In the comparison of the developmental competence from pronuclear stage oocytes into blastocyst stage embryos after Semen Cuscutae administration, that of the 4-week-old and 18-week-old (treated) groups showed significant increase, compared with that of the 4-week-old and 18-week-old (untreated) groups.

From these results, it was concluded that Semen Cuscutae has significant effects on the increase of the ovulation and the developmental competence from pronuclear stage oocytes into blastocyst stage embryos in mice and augments ovarian functions, the core of fecundity.

It is concluded that Semen Cuscutae can be applied in the improvement of fecundity in women and more continuous studies are expected in the future.

References