Sildenafil citrate decreased NK cell activity and enhanced chance of successful pregnancy in women with a history of recurrent miscarriage

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Implantation.

**Uterine cavity**
- lectin
- ECM
- IL-1, CSF-1

**Blastocyst**
- integrins
  - α5β1- Fn
  - αVβ3, α6β4
- LIF, EGF
- MMP-2, MMP-9;
- TIMPs

**endometrium**
- integrins
  - α1β1- Lam, C-IV
- estrogen
- progesteron
- Th1 (IL-2, TNF α, IFNγ)
- Th 2 (TGF-β, IL-3, IL-4, IL-5)
- NK CD 16+56-
- NK CD 16-56+

Shimada S, 2006; Perricone R, 2006; Yamada H, 2005; Wold, 2005
Quality of endometrium

◆ A typical trilaminar appearance with a minimum thickness of 7 mm and uterine artery pulsatility index of <3.0 are regarded as sound markers of endometrial receptivity. (Steer et al., 1992; Zaidi et al., 1995; Kelly et al., 2001)

◆ A trilaminar sonographic endometrial pattern of >8mm on day of HCG has been shown to be correlated with a high chance of pregnancy in patients being treated with IVF. (Gonen et al., 1990; Sher et al., 1991)

◆ Increased blood flow in the uterine arteries during menstrual cycle predict uterine arteries hemodynamic adaptation established during early pregnancy. (Bernstein et al., 2002)

◆ The presence of protodiastolic notch in the uterine arteries predict successful implantation. (Puerto et al., 2003)
Quality of endometrium

- The presence of sub- and intraendometrial vascularity on the day of transfer seemed to be mandatory for obtaining an ongoing pregnancy. (Maugey-Laulom et al., 2002)

- Endometrial and subendometrial vascularity are significantly reduced in women with unexplained subfertility during the mid-late follicular phase. (Raine-Fenning et al., 2004)
How to improve quality of endometrium?

- Oestrogens and low dose aspirin (LDA). (Sher et al., 1993; Weckstein et al., 1997) LDA 100mg significantly improved uterine blood flow velocity, implantation and pregnancy rate in IVF patients. (Rubinstein et al., 1999)

- Nitroglycerine, however unacceptably high rate of headaches, nausea and hypotension. (Smith and Brien, 1998)

- Combined pentoxifylline (800mg/day) - tocopherol (1000 IU/day) reduced fibroatrophic uterine lesions after childhood irradiation and significantly improved endometrial thickness and uterine artery flow. (Letur-Kornisch et al., 2002; Ledee-Bataille et al., 2002)
How to improve quality of endometrium?

- Sildenafil citrate (VIAGRA), a type 5-specific phosphodiesterase inhibitor, augments the vasodilatory effects of NO. Vaginal sildenafil (at a dosage of 25 mg, 4 times a day for 3-10 days) improves uterine artery blood flow and sonographic endometrial thickness. (Sher G, 2000, 2002; Paulus W, 2002; Kelly S, 2001)

- Viagra (50mg of sildenafil citrate administered orally 8-hourly from day 5 to ovulation) significantly increases subendometrial vascularity in apparently fertile women without effecting an increase in endometrial thickness or volume. (Raine-Fenning et al., 2003)

- Successful use of vaginal sildenafil citrate in two infertility patients with Asherman’s syndrome. (Zinger et al., 2006)
Viagra in recurrent miscarriage patients. Aim of the study

- Sildenafil citrate (VIAGRA) augments the vasodilatory effects of NO. Vaginal sildenafil improves uterine artery blood flow and sonographic endometrial thickness. While improving uterine blood flow in the proliferative phase, NO may have detrimental effects at the level of the endometrium during the implantation window. The NO-mediated release of cytokines such as tumour necrosis factor-α from activated natural killer cells has been implicated as a cause of implantation failure (Barroso et al., 1998). NK cytotoxicity has been reported to be predictive of subsequent pregnancy loss in women who had recurrent spontaneous abortions (RSA). Therefore, the purpose of the study was to establish the effect of sildenafil on NK cell activity in women with a history of RSA (after natural or IVF conception).
Thirty eight nonpregnant women with the history of RSA (at least 3 recurrent abortion) and thirty seven normal healthy women with the previous successful pregnancy outcome were studied. Measurement of uterine artery blood flow (pulsatility index, PI) was recorded using Doppler ultrasound by intravaginal probe in the study women. Natural killer cell activity was measured using flow cytometry. The following peripheral blood NK cells’ surface antigens: CD16, CD56 were also studied using flow cytometry (Simultest CD3/CD16+CD56, FACSCalibur, Cell Quest software, Becton-Dickinson). Administrations of sildenafil suppositories 25 mg were performed intravaginally four times per day during proliferative phase of the menstrual cycle in women with the history of RSA. NK cell activity before and after vaginal sildenafil therapy in RSA women were studied. In addition, influence of 10µg or 400ng sildenafil (400ng- physiological peripheral blood concentration of the drug) on NK cell activity after in vitro culture were performed.

Endpoints

Primary endpoints: NK cell activity in vivo (before and after sildenafil therapy)

Secondary endpoints: NK cell activity in vitro (after culture with sildenafil), uterine artery blood flow and endometrial thickness (before and after sildenafil therapy).
ULTRASOUND EXAMINATION (transvaginal probe GEM LOGIQ 400 CL L. 8.2-11.0 MHz, C. 3-4 MHz)

Uterine artery blood flow (pulsatility index, PI) and endometrial thickness was recorded during proliferative phase of the menstrual cycle in nonpregnant women with the history of RSA using Doppler ultrasound by intravaginal probe.

Management of RSA

Uterine artery blood flow in woman with the history of 8 RSA and increased number and activity of NK cells before Viagra therapy.
Management of RSA

ULTRASOUND EXAMINATION (transvaginal probe GEM LOGIQ 400 CL L. 8.2-11.0 MHz, C. 3-4 MHz)

Uterine artery blood flow (pulsatility index, PI) and endometrial thickness was recorded during proliferative phase of the menstrual cycle in nonpregnant women with the history of RSA using

Uterine artery blood flow in woman with the history of 8 RSA and increased number and activity of NK cells after Viagra therapy.
Natural killer (NK) cells’ surface expressions, NK cells’ activity and PI- uterine artery

Data are given as a mean ± standard error of the mean (SD)

<table>
<thead>
<tr>
<th>Study group</th>
<th>CD16 (%)</th>
<th>CD56 (%)</th>
<th>NK cells’ activity (%)</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal women (n=37)</td>
<td>12.39% (±5.66)</td>
<td>4.46% (±2.96)</td>
<td>8.29% (±5.00)*</td>
<td>2.37 (±0.36)</td>
</tr>
<tr>
<td>Recurrent miscarriage women (n=38)</td>
<td>13.16% (±5.9)</td>
<td>9.05% (±6.63)</td>
<td>15.67% (±11.86)</td>
<td>2.35 (±0.95)</td>
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</tbody>
</table>

*P<0.05. All data were analyzed using Mann-Whitney test.
NK cells’ activity in RSA group: before and after sildenafil therapy * $P=0.01$

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Minimum</th>
<th>Lower Quartile</th>
<th>Median</th>
<th>Upper Quartile</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std Dev</th>
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<tbody>
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<td>38</td>
<td>2.00</td>
<td>7.00</td>
<td>15.00</td>
<td>25.00</td>
<td>43.00</td>
<td>16.72</td>
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<td>1.00</td>
<td>5.00</td>
<td>8.00</td>
<td>13.00</td>
<td>63.00</td>
<td>11.28</td>
<td>11.64</td>
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**NK cells’ activity in RSA group: before and after sildenafil therapy, after in vitro culture with 10 μg sildenafil * P=0.02**

<table>
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<th>N</th>
<th>Minimum</th>
<th>Quartile</th>
<th>Median</th>
<th>Quartile</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std Dev</th>
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<td>4.00</td>
<td>7.00</td>
<td>-6.18</td>
<td>10.83</td>
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<tr>
<td>Before</td>
<td>19</td>
<td>0.00</td>
<td>4.00</td>
<td>9.00</td>
<td>20.00</td>
<td>30.00</td>
<td>11.95</td>
<td>9.50</td>
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<tr>
<td>After</td>
<td>19</td>
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<td>6.50</td>
<td>8.00</td>
<td>15.00</td>
<td>5.76</td>
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**NK cells’ activity in RSA group: before and after sildenafil therapy, after in vitro culture with 400 ng sildenafil * P=0.02**

<table>
<thead>
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<th>N</th>
<th>Minimum</th>
<th>Quartile</th>
<th>Median</th>
<th>Quartile</th>
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<td>8.00</td>
<td>20.00</td>
<td>33.00</td>
<td>12.42</td>
<td>9.86</td>
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<tr>
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<td>1.00</td>
<td>3.00</td>
<td>7.00</td>
<td>10.00</td>
<td>14.00</td>
<td>6.53</td>
<td>3.91</td>
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**Uterine artery PI and endometrial thickness in RSA group: before and after sildenafil therapy * P=0.05**

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Minimum</th>
<th>Quartile</th>
<th>Median</th>
<th>Quartile</th>
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<td>-0.67</td>
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<td>0.58</td>
<td>1.46</td>
<td>-0.12</td>
<td>1.00</td>
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<tr>
<td>P1 before</td>
<td>38</td>
<td>1.13</td>
<td>1.75</td>
<td>2.18</td>
<td>2.52</td>
<td>5.43</td>
<td>2.38</td>
<td>1.06</td>
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<tr>
<td>P1 after</td>
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<td>0.79</td>
<td>2.10</td>
<td>2.22</td>
<td>2.48</td>
<td>3.52</td>
<td>2.26</td>
<td>0.60</td>
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</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Minimum</th>
<th>Quartile</th>
<th>Median</th>
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<tbody>
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<td>-1.00</td>
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<td>2.80</td>
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<td>1.19</td>
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<td>Endo before</td>
<td>38</td>
<td>3.20</td>
<td>5.20</td>
<td>8.00</td>
<td>9.50</td>
<td>13.60</td>
<td>8.06</td>
<td>2.74</td>
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<td>Endo after</td>
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<td>6.00</td>
<td>7.00</td>
<td>9.00</td>
<td>11.00</td>
<td>13.70</td>
<td>9.25 *</td>
<td>2.23</td>
</tr>
</tbody>
</table>
Conclusions

- Our data suggest that sildenafil has no detrimental effect on NK cell activity, on the contrary NK cell activity was significantly decreased after vaginal Viagra therapy in the study women.
- Additionally, sildenafil significantly improves endometrial thickness.
- Therefore, vaginal sildenafil might be interesting therapeutic option for women with reproductive failure.
- This work was supported by grant nr 2 P05E 07926 from Committee for Researches (KBN).
All truths are easy to understand once they are discovered; the point is to discover them.

Galileo Galilei