The effect of sildenafil (Viagra™) on live birth and the development of mouse offspring

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Background

- Live birth rates following assisted reproduction now account for 1 to 3% of pregnancies in developed countries.
- Concerns regarding the safe use of assisted reproductive technology (ART) for the treatment of infertility have been voiced for several years, yet the vast majority of children conceived using these techniques appear normal.
- Low endometrial thickness is thought to be a major factor in ART therapies not resulting in a successful pregnancy.
- Sildenafil, a PDE5 inhibitor which relaxes the smooth muscles and increases blood flow, has been suggested as an adjuvant therapy to increase the endometrial thickness in women undergoing IVF.
- Nevertheless, there are serious concerns about the problems that sildenafil may cause to the developing foetus, since it can freely cross the placenta.

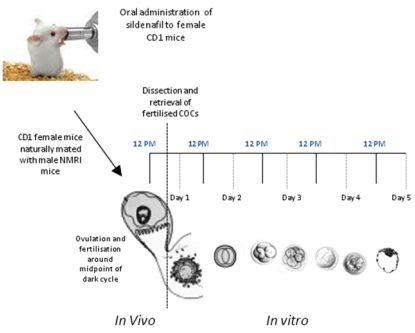
Objective

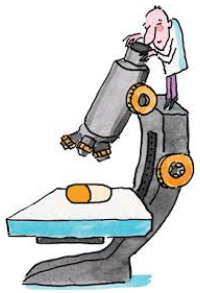
To investigate whether *in vivo* administration of clinically relevant doses of sildenafil to female mice prior to mating has any effect on the developmental capacity of the resultant embryos and live young.

Materials & Methods

➢ **Our approach is :**

- Compare implantation rates and foetal development after *in vivo* administration of different doses of sildenafil to untreated controls.
- Determine the number and gender of the offspring, and perform total body and organ allometry.





➢ Experiment ONE:

1- Mice were randomly assigned to either controls or three different doses of sildenafil (S1, S2,S3) administered by oral gavage twice daily over 2 days before mating then sacrifice.

2-Day one zygotes from each group were cultured in vitro until the blastocyst stage, then transferred into 2.5dpc CD1 pseudo-pregnant mice.

3- After pregnancy, all offspring were counted, sexed then sacrificed at 2 weeks after weaning for organ allometry (total weight, heart, liver and lungs, kidneys, spleen, pancreas

➢ Experiment TWO:

1-Same dose and administration regime as for Experiment 1.

2- Mated with male mice on morning of day 3 and allowed to carry pregnancy to term.

3- The number of offspring obtained were counted, gender was noted, and then at two weeks, the offspring were weighed then culled for individual organ weight (Allometry) and imprinting of gene analysis.

➢ **Statistical analysis:** Statistical analysis was performed by using 'R' Software. Two tailed P value of <0.05 indicates statistical significance.

➢ To decide whether gender was determined by treatment concentration, binomial logistic regression models were fitted in 'R' and changes of deviances over the changes in df in models were compared to the chi square table.

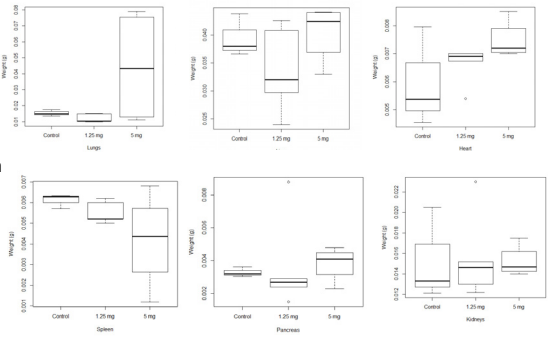
Results

➢ Experiment ONE:

◆Table 1. Number of blastocysts transferred and offspring for each treatment group

Treatment Group	S1 (1.25mg/Kg)	S2 (2.5 mg/Kg)	S3 (5 mg/Kg)	Control
Total number of blastocysts transferred on the day 5	17	10	21	13
Total number of offspring	5	0	4	3
Pregnancy success rate(%)	29.41	0	19.04	23.07
Mean Weight	13.352	0	13.25	11.19

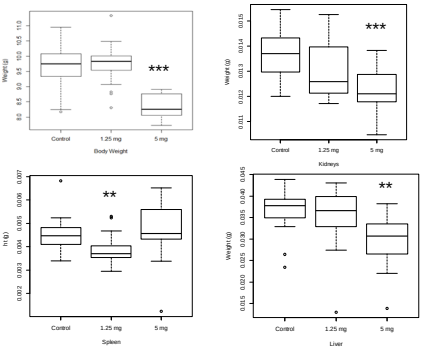
◆Figure 1. **Box plots presenting the median values for different organs: Lungs, Heart, Liver, Spleen, Pancreas and Kidney, in the first experiment (No significant differences) were detected . P>0.05)**



◆Table 2. Number of blastocysts transferred and offspring for each treatment group

Treatment groups	S1(1.25mg/kg)	S2(2.5mg/kg)	S3(5mg/kg)	Control
Number of female mice mated	10	10	10	10
Number of female mice pregnant	2	0	1	2
No of live offspring on day 14	21	N/A	12	23
No of offspring per pregnant female	10.5	N/A	12	11.5
Mean weight (g)	9.732	N/A	8.358	9.657

◆Figure 2. **Box plots presenting the median values for body weight and organs: Lungs, Heart, Liver, Spleen, Pancreas and Kidneys for different treatment concentration in the second experiment. Asterix indicate significant difference from control.**



Discussion & Conclusion

➢ In both experiments, we found that there were no live births from 10 transfers (Experiment one) or 10 matings (Experiment two) in mice treated with 2.5mg/kg sildenafil. Hence this dose, despite being in the normal physiological range, appears to be detrimental, although mechanism unknown. (Table 1, Table 2)

➢ The first experiment resulted in no significant differences in any of the organ weights or the mean body weight when embryos transferred into recipients (Table 1, Fig. 1; P>0.05).

➢The second experiment revealed negative effects of sildenafil in terms of body weight and kidney, spleen and liver size (Fig. 2) when treated mice were allowed to carry pregnancy to term. There were no significant effects on the size of pancreas, heart and lungs or gender ratio (Ratio of F/M in Control: 43.5/56.5, S1: 61.9/38.1, S3: 33.3/66.7).

In conclusion: These preliminary findings indicate that sildenafil treatment prior to conception may have negative effects on subsequent embryo and fetal development and that these effects may be mediated by effects on both the embryo and the uterine environment. Further work elucidating the mechanisms of sildenafil metabolism and action in the mouse and human models is needed.