

ULTRASTRUCTURAL EFFECTS IN LUTEAL CELLS AFTER PHOSPHODIESTERASE-5 INHIBITOR TREATMENT

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Adenosine 3',5'-cyclic monophosphate (cAMP) and guanosine 3',5'-cyclic monophosphate (cGMP) are ubiquitous nucleotides that, in concert with calcium and IP₃, orchestrate intracellular signaling. Cyclic nucleotides cGMP and cAMP have different effects depending of the cell type. In the ovary, the role of cAMP is well known, and the actions of LH and FSH on ovarian functions are believed to be mediated in large part through its increased production. On the other hand, cGMP concentrations are regulated in an inverse manner compared to cAMP in the ovary, in certain phases of cycle [1]. Many studies have reported effects of cGMP on ovarian cells, suggesting that cGMP may be a necessary component as both a growth enhancer and a survival factor [2, 3]. The use of phosphodiesterase-5 inhibitor (iPDE5) increases intracellular cGMP levels. The first orally administered iPDE5 medication was Sildenafil, subsequently, pharmaceutical companies developed further iPDE5 drugs, such as Vardenafil and Tadalafil. Vardenafil was the second selective iPDE5 marketed and is the most potent iPDE5 inhibitor on the market. In the present study, we attempted to evaluate use of Vardenafil on ovary cells.

Twenty-four adult female *Swiss webster* mice, 45-day-old, were used in all experiments. One experimental group composed by twelve animals received 5mg/kg body weight of Vardenafil for 30 days per os in aqueous solution in water bottle. The control group also composed by twelve animals received only pure water. After treatment the experimental and control animals were killed and pieces of ovary were quickly excised with a scalpel and fixed for electron microscopy evaluation. Serum was separated and stored at -70°C for biochemical analyses.

Luteal cells from control group showed lipid droplets and mitochondria with tubular cristae. Ribosomes were visible near to the nucleus (Figure 1A and 1B). Luteal cells from vardenafil-treated group showed numerous mitochondria, and paucity of lipid droplets, comparing to control group. Several ribosomes were present in cytoplasm, scattered or attached to the endoplasmic reticulum (Figure 1C and 1D). Size of luteal cells seemed to be also different. To certify, we measured the medium diameter of 40 cells (20 for each group), and compared groups. Mean diameter of Vardenafil-treated group was considerably smaller than control cells. Also, Vardenafil-treated cells had statistically significant lower number of mitochondria and lipid droplet (Table 1). Biochemical analyses showed that Vardenafil diminished serum concentrations of HDL. Conversely, serum levels of LDL, VLDL, cholesterol, triglyceride and progesterone presented no significant differences between groups (Table 2).

Despite morphological changes as minor lipid and mitochondria content suggested a diminished steroidogenesis, biochemical analyses showed no differences in the progesterone serum levels. Subsequently, morphological changes could be related to lower levels of HDL due to treatment with Vardenafil, since HDL have been proved as major cholesterol source in luteal cells [4]. Other studies observed that *knockout* mice for HDL receptor showed ovaries with a decrease of lipid content on luteal cells; although serum progesterone showed normal levels comparing to *wildtype* mice [5]. Similarly, the present results showed no difference in the serum progesterone levels in Vardenafil-treated animals; however, long-term treatments could be useful for more detailed data.

We can then suggest that the phosphodiesterase-5 inhibitors can interfere in luteal cells by two different mechanisms: directly by increasing the concentration of intracellular cGMP intracellular or by altering the HDL-cholesterol levels.

References

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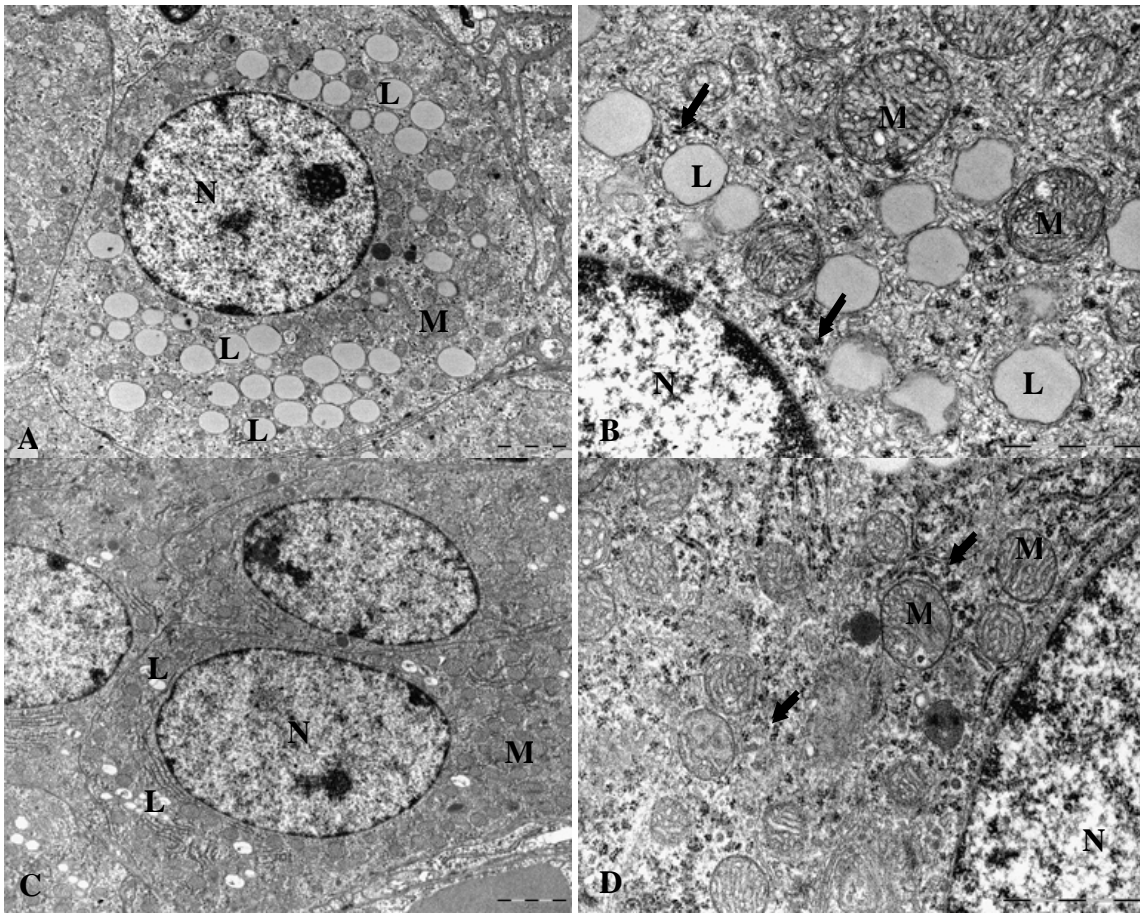


Figure 1 - Ultrathin sections of control luteal cells (1A and 1B) and vardenafil-treated cells (1C and 1D). In control group, numerous lipid droplets (L) and mitochondria (M) with tubular cristae are scattered through the cytoplasm. Ribosomes (arrows) were evident nearby to the nucleus (N). In vardenafil-treated group, observe numerous mitochondria (M), and few lipid droplets (L), compared to control group. Several ribosomes were present in cytoplasm (arrows), isolated or attached to the endoplasmic reticulum. Bars, 2 μm (A, B); 1 μm (C, D).

	Control	Vardenafil-treated
Mean diameter	13,088 \pm 1,538 μm	10,638 \pm 1,386 μm *
Mitochondria	105,1 \pm 37,087	63,35 \pm 23,979**
Lipid	54,55 \pm 27,53	24,15 \pm 12,38***

Table 1 - Ultrastructural morphometric analyses after Vardenafil treatment. Significant difference between Vardenafil 5mg/kg and control samples: *mean diameter ($t(0,05; 38)=5,2921$; $p<0,0001$). **mitochondria ($t(0,05;38)=4,228$; $p<0,0001$). ***lipid droplets ($Z(U)(0,05;38)=3,828$; $p<0,001$).

	Control	Vardenafil-treated	P<0,01
Progesterone (ng/dL)	0,97	1,33	0,00098
Cholesterol (mg/dL)	73,5	70,11	0,178
Triglyceride (mg/dL)	213,7	198,32	0,174
VLDL (mg/dL)	42,53	39,56	0,167
LDL (mg/dL)	6,14	5,23	0,206
HDL (mg/dL)	75,85	63,48*	0,173

Table 2 - Effect of Vardenafil treatment on mice serum progesterone, cholesterol, triglyceride, VLDL, LDL and HDL levels. *Significant difference between treated and control biochemical samples ($p<0,01$).