

Research strategy of the therapeutic quality of mud and salty water from Bear Lake - Sovata

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Infertility (sterility) is a health problem affecting 15% of couples of reproductive age. Today only a few are known about causes and treatment options involved in the pathology of infertility, while a number of issues remain unknown. Currently natural therapeutic factors from Sovata are used, based on experience over the years, for the following causes of infertility: tubal obstruction, laparoscopic postneosalpingostomie recovery treatment (prevention of restenozations), sequelae after pelvic inflammatory disease, peritonitis, post-inflammatory adhesions; secretory ovarian dysfunction (estrogen, progesterone), polycystic ovarian syndrome, endometriosis, infertility induced by stress.

Clinical data suggest that peloidotherapy is useful in preparing the uterus for in vitro fertilization, but without scientific evidence. Mud and water from Bear Lake must be analyzed, both in terms of physico-chemical, microbiological and in terms of phyto-and zooplankton to detect organisms that grow in the lake and are structural and biological determinants of sludge role in studies on infertility. Previous studies conducted at the Institute of Balneology, Bucharest, have suggested the existence in the lake water of certain substances having similar effects on the endometrium as the female sexual hormones. These substances, with a natural source is probably the result of the presence of different species (including *Artemia salina*), in the plankton composition of Bear Lake water. After identifying the

groups of indigenous microorganisms and plankton composition that provides therapeutic properties, is expected to obtain and characterize a new sapropelic mud extract to demonstrate, through experimental studies of physico-chemistry, biology, microbiology, cell biology, histology, endocrinology, gynecology and molecular biology, the therapeutical capacity of a new mud extract in the infertility treatment.

Are planned experiments on laboratory animals (Wistar rats / rabbits New Zealand) taking experimentally induced infections with low levels of fertility induced by various methodologies including genital swelling controlled or pharmaceutical interventions on endocrine control. Animals with induced infertility will be treated experimentally by parenteral injections with the mud extracted from Bear Lake, Sovata, and on the other hand will do similar procedures to those used now to Sovata with natural therapeutic factors. Obtaining of these animal models for infertility is a new approach to clinical trials. In this way we intend to quantify the therapeutic effects of mud extract in the the treatment of infertility. Also, in this way we can separate placebo effects from those of spa procedures and distinguish them from the therapeutic effects attributable to mud and water from Bear Lake. Clinical research will be accompanied by biomedical and molecular determinations in vitro, using this time as an experimental model the primary cultures of endometrial cells obtained by harvesting in

patients with established clinical infertility, in compliance with all legal rules of ethics. The analyzes at cellular and molecular level allow monitoring of changes on various intracellular signaling pathways, particularly prostaglandin signaling pathway. Data will be compared with those obtained from *in vitro* human epithelial cell line, purchased during project implementation.

The experimental results obtained will allow the scientific validity of the method of infertility treatment proposed, with new mud extract. Experimental data *in vitro*, using a liquid membrane system (hexane phase source / bovine serum / final phase of hexane), shows that the rate of absorption of steroids from the blood in the the intracellular space is controlled, not only in free form, but also in the form bound to plasma proteins such as albumin. It is now generally accepted that steroid fractions that bind with high affinity to plasma proteins such as globulins are more readily available to tissues. Recent studies have shown that, in the addition to free steroids, those bound to albumin in plasma may be also available to tissues. Steroid binding of albumin is affected by temperature, pH, competing sources of fatty acids and ligands. Albumin is the most abundant protein in the circulatory system, representing 60% of total serum protein. To mammals, albumin is synthesized by the liver and has a circulation half-life of 19 days. Its main function is to transport fatty acids, a variety of metabolites and drugs such as anticoagulants, tranquilizers and anesthetics. In general *in vitro* bioassays are valuable tools for screening environmental samples for the presence of bioactive substances (e.g. disturbing the endocrine system).

Enzymes are vital activators in the body processes, also in the mud they are known to play an important role in maintaining soil health and its environment. These enzymes include amylase (EC 3.2.1), arylsulphatase (EC 3.1.6.1), β -glucosidase, cellulase (EC 3.2.1.4), chitinase, dehydrogenase, phosphatase (EC 3.1.3), protease, and urease released from plants (Miwa et al. 1937). Enzyme activity in

the mud is mainly of microbial origin, being derived from intracellular enzymes, cell-associated or free. A unique balance of chemical, physical and biological components (including microbial activity, particularly enzymatic), contribute to maintaining the health of mud.

Mud health assessment needs therefore, indicators of all these components. Sludge healthy is essential for the integrity of terrestrial ecosystems to remain intact or to recover from disturbances such as drought, climate change, pest infestation, pollution and human exploitation (Ellert et al. 1997). Enzyme levels in the mud systems vary in the amount due to the fact that each type has different amounts of soil organic matter composition and activity of living organisms and the intensity of biological processes (Stevenson, 1986). The occurrence of estrogens in the aquatic environment attracts attention because of their potential endocrine highly disturbing. Steroidal estrogen hormones are molecules structurally based on phenanthrene ring. They are produced from cholesterol primarily in the ovaries in response to signals from the brain or other organs and, even if in smaller quantities in the testes of men. Estrogens found naturally for all classes of vertebrates are 17β -estradiol type (β -E2), 17α -estradiol (α -E2), estrone (E1) and estriol (E3). Estrogens play a role in sexual development and are responsible for the development of female secondary sexual characteristics, control reproductive cycles and fertility (Tyler and Sumpter, 1998). In addition, they also function in the nervous system, circulatory system and to regulation of bone density (Sandberg, 2002).

Chemical substances known to have potential to bind and activate estrogen receptors are natural compounds such as phytoestrogens, and synthetic estrogen mycoestrogens analogues, such as pharmaceutical highly 17α -ethynylestradiol (the active ingredient in contraceptive drugs).