COMPLEMENTARY THERAPY IN POLYCYSTIC OVARY SYNDROME

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Abstract – Polycystic Ovary Syndrome (PCOS) is an endocrine disease. PCOS afflicts 5 to 10 % of women of reproductive age. The symptoms are: amenorrhea, oligomenorrhea, hirsutism, obesity, infertility, chronic hyperandrogenic anovulation and acne.
Other risk factors aggravate this condition: insulin resistance, obesity, hypertension, dyslipidemia, inflammation and subclinical cardiovascular disease.
Anxiety, depression and reduced quality of life are also common.
This review highlights the mechanisms and the beneficial effects of acupuncture, exercise and resveratrol on animal models and on humans affected by PCOS.

Keywords: PCOS, acupuncture, exercise, resveratrol.

I. INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is an endocrine disease characterized by a still unclear aetiology resulting from the interaction among genetic, behavioral and environmental conditions and where ovarian hyperandrogenemia plays a key role [1].
Indeed, several studies demonstrate that a low dose of testosterone in female monkeys Rhesus increases luteinizing hormone (LH) secretion [2, 3, 4, 5]. Further, androgens can determine PCOS-like syntomatic pictures in animal female and female-to-male transsexual models [6].
Hyperinsulinemia/insulin resistance and abdominal obesity are relevant. However, whether hyperandrogenism results from the hyperinsulinemia of insulin resistance or vice versa is unclear [7, 8]. Studies performed on monkeys, sheep and rats demonstrate that prenatal androgenization is a cause of PCOS [9, 10]. In human, only one study has verified the presence of testosterone in the blood of babies from PCOS mothers [11]. Also the exposition to androgens in a prepuberal period causes PCOS in animal models [12, 13]. At the begin of ovary development, antimůllerian hormone (AMH) is a determinant of follicular activity. In rats increased AMH, as in PCOS, decreases the follicular growth in ovary [14, 15] and is linked with low levels of FSH [16, 17]. Moreover, as shown from in vitro studies on granulose cells of small bovine follicles cultured with testosterone, AMH expression is downregulated and may cause PCOS [18].
It is known that neuroendocrine defects can increase the persistently rapid LH pulsatility- and this latter have been linked with augment of the ovarian volume, several antral follicles and androgen hyperproduction [8]. The high activity in the sympathetic neurons innervating the ovaries precedes the development of ovarian cysts in rats [16]. Women with PCOS may have increased ovarian nerve fiber density and androgen hypersecretion. Further, the high circulating testosterone in PCOS women explains their high sympathethic nervous system activity [17] associated with several clinical findings illustrated in Table 1 [19].

TABLE 1. SIGNS AND SYMPTOMS OF PCOS CAUSED BY INCREASED ACTIVITY IN SNS

Hyperandrogenism
Increased sympathetic nerve activity
Altered GH/IGF-1 axis
Hypersecretion of LH
Anovulation
Visceral obesity
Hypertension
Insulin resistance
Psychological stress

LH, Luteinising hormone; GH, growth hormone; IGF, insulin growth factor.

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It has been demonstrated that PCOS women have ovarian theca interna hyperplasia responsible for the increased androgen steroidogenesis. Besides, theca cells have higher expression of LH receptors with augmented sensibility to LH stimulation [18, 20, 21].
GnRH pulse generator is inhibited by the central β-endorphin system that also modulates sympathetic tone. Diminished or augmented β-endorphin and dopamine

activity causes tonic increasing release of LH [22, 23]. High plasma β-endorphin levels are related to hyperinsulinemia, stress [24, 25] and also to PCOS. Growth hormone (GH) and insulin growth factor-1 (IGF1) regulate sympathetic nerve tone : and in PCOS have been shown to be present disturbances in the somatotrophic axis (GH/ IGF-1) [26]. Moreover, high sympathetic nervous tone is the most relevant risk factor for heart diseases and mortality [27, 28]. The effects of these alterations are shown in the figure 1 [29, 30 , 4].

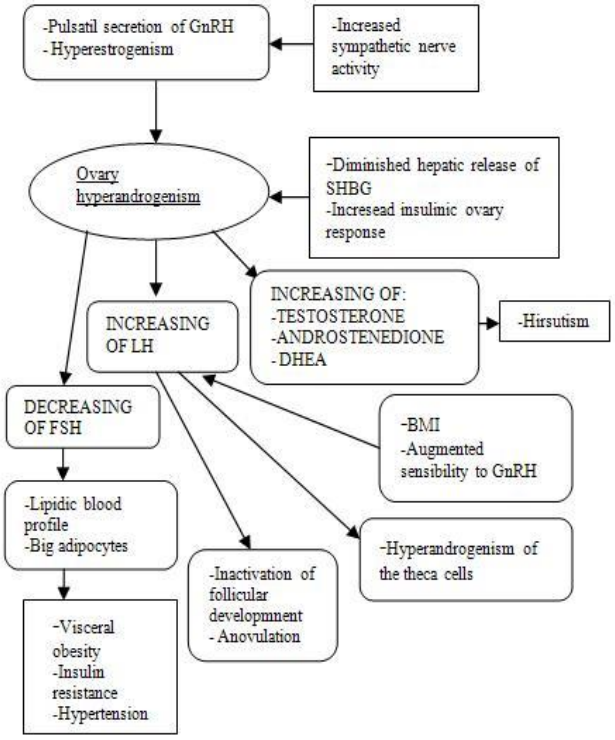

Fig. 1: Schematic illustration of PCOS events.
Aquino C. I. , 2013

II. METHODOLOGY

1. ACUPUNCTURE

Because PCOS is a multifactorial pathology several treatments (see table 2) have been proposed [31]. It is demonstrated that cognitive performances (word learning test and symbol digit substitution test) are also improved significantly after the treatment (Metformin) for PCOS [32]. Acupuncture is now widely used in the Western population accounting for one in three Americans using it [5].

-OLD THERAPY
Oral contraceptives (OC)
Anti-androgens
-NEW THERAPY
Insulin sensitizers:
(Metformin Thiazolinediones)
-EMERGING THERAPY
Statins
Acupuncture
Dietary products and nutrients.
Vitamin D
Herbal medicines
Vitamin B12 and folate.
Resveratrol
AGEs low diet
Phisical activity

Acupuncture is an ancient beneficial practice that use needles for manual or electrical sensory stimulation of somatic afferent nerves. These nerves innervate the skin and muscles.
Auricular stimulation is widely used to improve pregnancy (see table of auricular acupuncture in figure 2).
Traditional Chinese medicine (TCM) affirmed that acupuncture restores the balance between Yin and Yang. In the modern interpretation of the Western Medicine, acupuncture restores the equilibrium between parasympathetic and sympathetic activity [33].
The insertion of needles determines a specific pattern of afferent response in peripheral nerves stimulating the spinal cord and the central nervous system [34].

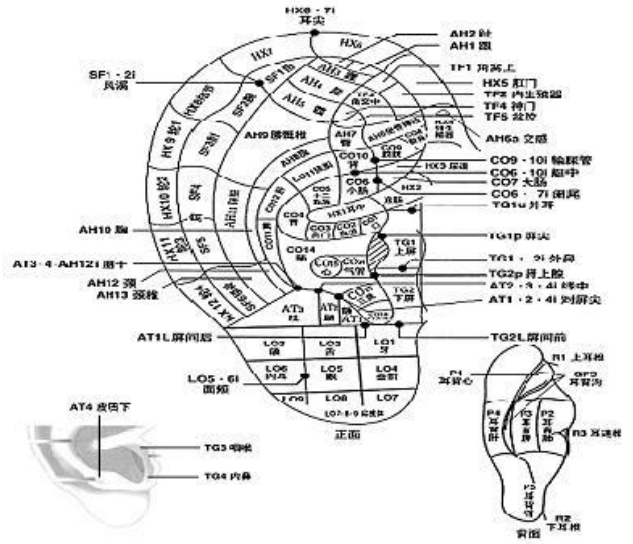

Fig.2: Auricular acupuncture.
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Electroacupuncture (EA) takes advantage of a low frequency (1-15 Hz) electrical stimulation that elicits

TABLE 2. TREATMENTS OF PCOS

muscle contraction and augments the analgesic effects of acupuncture [35]. Acupuncture can activate autonomic responses (as seen in figure 3), the descending inhibitory pain control systems, and the propriospinal heterosegmental antinociceptive C-fibers [36, 37] and Aα, β, δ fibers [38, 34]. Positive results of this millenary treatment for PCOS women is related to: the central sympathetic inhibition through a major release of endorphin, the change of uterine blood flow and motility, the stress reduction. Acupuncture regulates the hypothalamic-pituitary-ovarian axis (HPO) actions by modulating central opioids, in particular β-endorphin [38, 39] in a context of mesencephalic and brainstem network [40, 41].

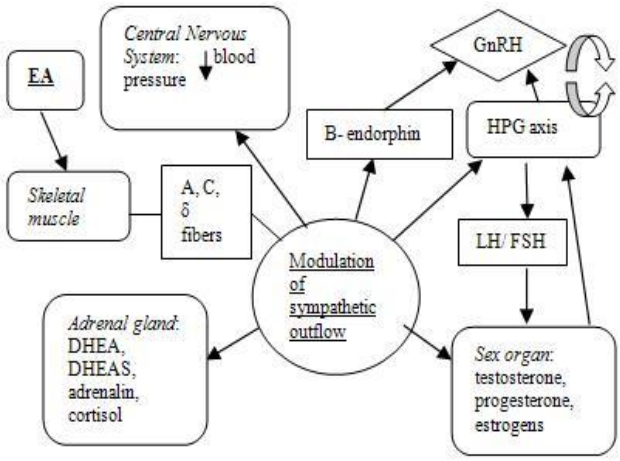

Fig. 3: EA and autonomic effects.
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There are opioid receptors of several types: μ, δ and κ in peripheral afferent nerve terminals [42, 43]. β-endorphin is a derived from proopiomelanocortin (POMC). It is produced in the arcuate nucleus of hypotalamus and in the pituitary gland and binds μ receptors [44, 45, 46]. This nervous network is composed by: hypothalamus, medulla oblongata, ventrolateral periaqueductal gray, and the dorsomedial prefrontal cortex. This functional circuit appear to be very important for the autonomic regulation [47, 48], in particular for the pain modulation. Further, functional MRI and PET studies demonstrate that using acupuncture is possible to show significant modulatory effects on the limbic system, paralimbic and subcortical gray structures [49 , 50, 51, 52, 53, 38].
An important acupuncture point, used also in PCOS and in other disorders, is ST 36 (stomach 36 or Zusanli; shown in figure 4) on the lower leg .

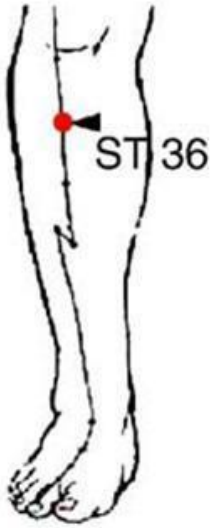

Fig. 4: localization of ST36 in the tibialis anterior muscle, 4 fingerbreadths below the kneecap and 1 fingerbreadth
lateral from the anterior crest of the tibia [54].
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When it is stimulated, fRMN shows a predominance of signal attenuation, particularly in the limbic/paralimbic regions of the telencephalon, diencephalon and brainstem [50, 51, 53].
This evidences an integrated response of the human cerebro-cerebellar and limbic systems to acupuncture treatment. As shown also in rats models, after acupuncture, fRMN and PET results indicate an integrated response from multiple functional units of the brain which appear to be dependent on the psychophysical status .
Moreover, the hemodynamic response correlates with acupuncture effects on the monoaminergic systems. Maybe these neuromodulators, in particular dopamine, may play an important role in acupuncture actions [54].
Another important beneficial effect of acupuncture is related to the actions on the corticotrophin releasing hormone (CRH)-produced by the hypothalamus.
β-endorphin and reproductive functions are linked to GnRH and LH release.
Estrogens are important to ovulate for the LH surge [55] and opioids inhibits the effect of estrogens [56].
CRH is released under stress conditions and decreases GnRH secretion. Acupuncture decreases the levels of CRH and modulate the HPO and HPA axis [57].
Moreover, acupuncture have been shown to increase glucose uptake and microcirculation [58, 59].
Needles stimulations decrease sympathetic activity and increase parasympathetic activity inhibiting the dorsomedial prefrontal cortex [60]. Peripheral nerves release neuropeptides: neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP), substance P and calcitonin gene-related peptide (CGRP) with a rapid reaction [61, 62].
EA stimulates enkephalinergic neurons in the brain areas that regulates sympathetic outflow [63, 64].

According to these results, Li et al. [65] found that a single brief acupuncture stimulation at points P5-P6 transiently induces the production of enkephalin associated with a brain regulation of the sympathetic outflow.
Consequently, acupuncture may control the hypothalamic-pituitary-adrenal (HPA) axis (diminishing cortisol concentrations) and the hypothalamic-pituitary- gonadal (HPG) axis (modulating central β-endorphin production and secretion) [66]. EA controls the symphatetic reflex responses by decreasing the release of GABA in the ventrolateral periaqueductal gray (vlPAG) [67]. The decrease of GABA disinhibits vlPAG cells. This action modulates the activity of rostral ventrolateral medulla (rVLM) neurons attenuating the sympathoexcitatory reflex responses [68]. Probably, both glutamate and GABA are involved in the mechanism of acupuncture modulation of autonomic response.
Moreover, EA could give benefits to PCOS and other metabolic disorders, by altering sympathetic signaling [69, 70]. EA reduces $mRNA^{NGF}$ in dihydrotestosterone (DHT) -induced rats and also in estradiol-valerate – (EV-) induced rats which have been used as PCOS models [71]. Nerve growth factor (NGF) is, in fact, higher in PCOS women ovaries and in EV-induced rats PCOS models [72, 73, 74]. NGF arrests follicle growth, augments ovarian sensibility to gonadotropins, increases adrenergic receptors and it causes ovarian hyperinnervation in transgenic mice models generated by pronuclear microinjection of the transgene construct into fertilized eggs from B6D2F1/J mice followed by transfer of the injected eggs into the uterus of a surrogate mother [72].
The decrease of NGF expression in peripheral organs could improve EA by modulating the autonomic nervous system activity [75]. In rats, EA decreases circulating testosterone concentrations and modulate the expression of opioid receptors μ and κ in the hypothalamus [76].
Furthermore, acupuncture in rats normalizes estrous cyclicity [76, 77], improves ovarian morphology [70], reduces circulating concentrations of inhibin B [78] and increases progesterone levels [76].

2. PHYSICAL ACTIVITY AND RESVERATROL

As shown in the figure 1, PCOS is characterized by lipidic blood profile, bigger adipocytes–and insulin resistance. These alterations determine visceral obesity and hypertension [79, 80]. Insulin resistance in skeletal muscles is related to alterations in fatty acid metabolism and mitochondrial abnormalities. In PCOS women it is associated with reduced gene expression of peroxisome proliferator-activated receptor γ coactivator α (PGC-1α) and sirtuins (SIRT) 1 and 3 [81, 82].
Resveratrol, a natural polyphenol, may have positive metabolic effects. It balances lipid profile, decreases adiposity and improves insulin sensitivity [83]. Resveratrol increases, in skeletal muscle, the mRNA levels of estrogen-related receptor α (*Erra)*, PGC-1α, and the ERRα/PGC-1α signaling pathway target gene expression of nuclear respiratory factor 1 *(Nrf1)* (all decreased in PCOS) [84, 85].
Resveratrol causes apoptosis and diminishes proliferation of rat theca cells, reduces androgen levels by arresting Cyp17a1 gene expression [86, 87]. Some of these results are linked to the activation of SIRT 1 [88]. Resveratrol can activate AMP-activated protein kinase (AMPK) using a cAMP signaling and mediates glucose transporter (GLUT)-4 translocation and higher glucose uptake [89]. High leptin levels inhibit ovulation in rats [90] : this polyphenol combined to physical activitity reduce fat mass and also leptin [69, 91]. Moreover,
physical activity and somministration of resveratrol improve estrus cyclicity in PCOS rats [92]. The study by A. Benrick et al. by treating PCOS rats with resveratrol demonstrates that resveratrol has beneficial effects on adiposity (that improves) and cyclicity in a similar manner to exercise, but it is not a good candidate for treating insulin resistance associated with PCOS [92].
Moreover, physical activity and acupuncture decrease high muscle sympathetic nerve activity in women affected by PCOS. It is also demonstrated that acupuncture is more effective than physical exercises [18,93]. Physical activities determine weight loss improving cardiovascular order and insulin cellular response; visceral adiposity and insulin response (IR) decrease [94, 95].
Regular and moderate aerobic exercises (90 min per week at 60-70 % VO2max) over a short period improve ovulation and menstrual frequency [96, 97].

III.RESULTS

Goal of these review is to make a transactional correlation between the results obtained in PCOS animal models and human using acupuncture, physical activities and resveratrol. In the tables 3, 4 and 5 some of the experiments performed in this field are compared.

TABLE 3: EFFECTS OF ACUPUNCURE ON RODENTS MODELS

	References	Description	Results
1	L. Manni, et al. 2005.	Virgin adult Wistar Kyoto rats were divided into four experimental groups: i) an Oil group (*control*, n = 8), ii) an Oil group receiving EA (*EA*, n = 8), iii) a PCO group (*PCO*, n = 8), and iv) a PCO group receiving EA (*PCO+EA*, n = 8). Daily stimulation were bilateral in the mm. biceps femoris and erector spinae, in somatic segments corresponding to the innervation of the ovaries.	EA modulated positively mRNA of PCOS rat models
2	J. Johansson et al., 2013	In rats with DHT-induced PCOS intense (5 days/wk) low-frequency EA for 4–5 wks .	Manual stimulation of acupuncture needles has a greater effect on glucose tolerance
3	E. Stener-Victorin et al., 2000	12 treatments by analyzing NGF in the central nervous system and the endocrine organs. Higher concentrations of NGF were found in the ovaries and the adrenal glands in the rats than in the control rats.	EA inhibits hyperactivity in the sympathetic nervous system.

TABLE 4: EFFECTS OF ACUPUNCTURE ON HUMANS WITH PCOS

	References	Description	Results
4	J. Johansson et al., 2013	Manual acupuncture with EA (2Hz) using needles placed in abdominal and leg muscles in the same somatic segment as the innervations of the ovaries. The treatment period was 16 weeks and women received in total 14 treatments.	Improvement of: menstruation pattern, LH/ FSH ratio, estrogen, and testosterone
5	E.Stener-Victorin et al., 2000.	EA is used on 24 anovulatory women with PCOS twice a week for eight times when compared with the baseline values. Moreover, acupuncture over the acupoint LI4 was reported to inhibit uterine motility.	The percentage of ovulatory cycles increased 66% up to 3 months after acupuncture

TABLE 5: EFFECTS OF RESVERATROL AND EXERCISE ON HUMANS AND RATS WITH PCOS

	References	Description	Results
6	A. Benrick et al., 2013	Female Wistar rats: Resveratrol was administrated 5 days a week for 4weeks and for all 7 days of the final treatment week. Rats in the exercise group had free access to a running wheel for 5wks .	*Exercise and Resveratrol treatments decreased adiposity, improving estrus cyclicity in PCOS rats. Exercises is more effective than resveratrol.*
7	E. Stener-Victorin et al., 2009.	84 women were randomized: EA (n _ 33), physical exercise (n _ 34), or untreated control (n _ 17) group.	EA and physical exercise reduced high sympathetic nerve activity in PCOS.

IV. DISCUSSION

In the experiment no.1 Manni et al. demonstrated that repeated low-frequency EA treatments modulate the expression of mRNA and the amount and distribution of proteins of α1-, and β2-adrenoreceptors (ARs), and of p75NTR in rats with steroid-induced PCOS [71].

In the study no.2 electrical and manual muscle stimulation affect glucose homeostasis through different mechanisms in rats with DHT induced PCOS. Manual stimulation improved whole-body glucose tolerance, an effect that was not observed after electrical stimulation, but did not affect molecular signaling pathways to the same extent as electrical stimulation. Although more functional signaling pathways related to insulin sensitivity were affected by electrical stimulation, maybe manual stimulation of acupuncture has a greater effect on glucose tolerance [78].

In the experiment no.3 E. Stener-Victorin et al. demonstrated high sympathetic activity in PCOS model rats and its decrease after EA treatment [73] .

In the study no.4 Johansson et al. addressed acupuncture as a potential treatment option in women with PCOS. Several studies indicated in fact that acupuncture is beneficial for ovulatory dysfunction in PCOS. This is also related to decreased levels of sex steroids and inhibin B. Although the clinical data does not support changes in LH pulsatility/secretion pattern as a possible mediator for this effect, there is strong evidence of central components that probably involve both opioid and sympathetic activities, an effect that may be mediated via the androgen receptor [78].

In the experiment no.5 Stener-Victorin et al. demonstrated that the mean pulsatility index (PI) of uterine vessels was significantly reduced shortly after electroacupuncture. This may improve the endometrial environment for embryo implantation [73] .

In the study no.6 A. Benrick at al. performed a 5-6 weeks resveratrol treatment on PCOS rats and did not find improvements in insulin sensitivity in DHT-induced PCOS rats, while exercise restored insulin sensitivity to a similar level as in control rats. Physical exercise also had beneficial effects on fat mass, adipocyte size, and estrus cyclicity [92].

In the experiment no. 7, it is evidenced that low-frequency EA *and* physical exercise decrease high sympathetic nerve activity in women with PCOS. Thus, since muscle sympathetic nerve activity (MSNA) can increase the cardiovascular risk, treatment with low-frequency EA or physical exercise aimed to reduce MSNA may be of importance for women with PCOS [98].

The effects of acupuncture on NGF expression are summarised in figure 5 [71, 73, 74, 99].

V. CONCLUSION

Several studies confirmed that acupuncture, physical activity and (to a lesser extent) resveratrol may improve metabolic, hormonal and psychological profile of PCOS in women. These positive results have been shown also in animal models, underlying the importance of these treatments, and their combinations, in clinical applications.

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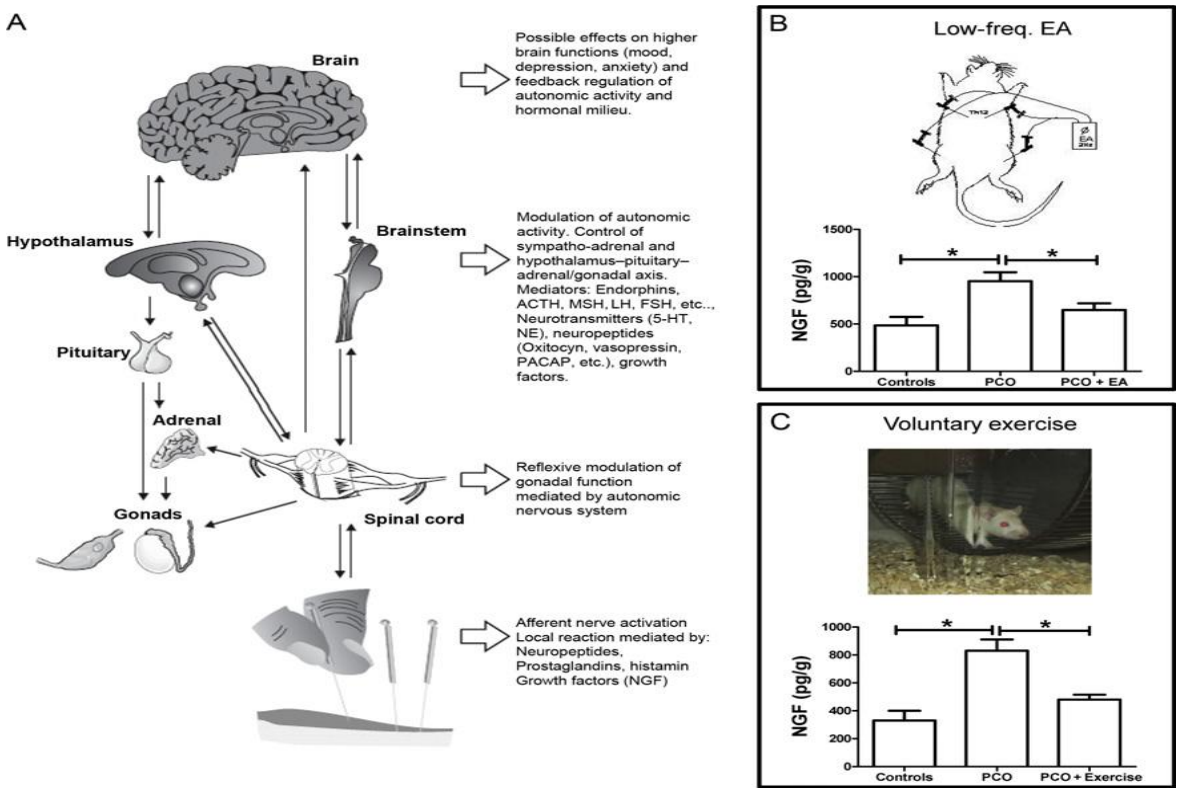

Fig. 5 Neurophysiological effects of acupuncture (A), EA (B) and physical activity (C) in rats affected by estradiol-induced polycystic ovaries
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(B) parts of this figure were originally published in Stener-Victorin et al. (2003);
(C) part of this figure was originally published in L. Manni Ph.D. thesis (ISBN: 91-628-6484-X).

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