Centro Salute Donna
Azienda USL Ferrara

OSTETRICIA e GINECOLOGIA

2022

8,9 aprile 2022

Hotel Astra

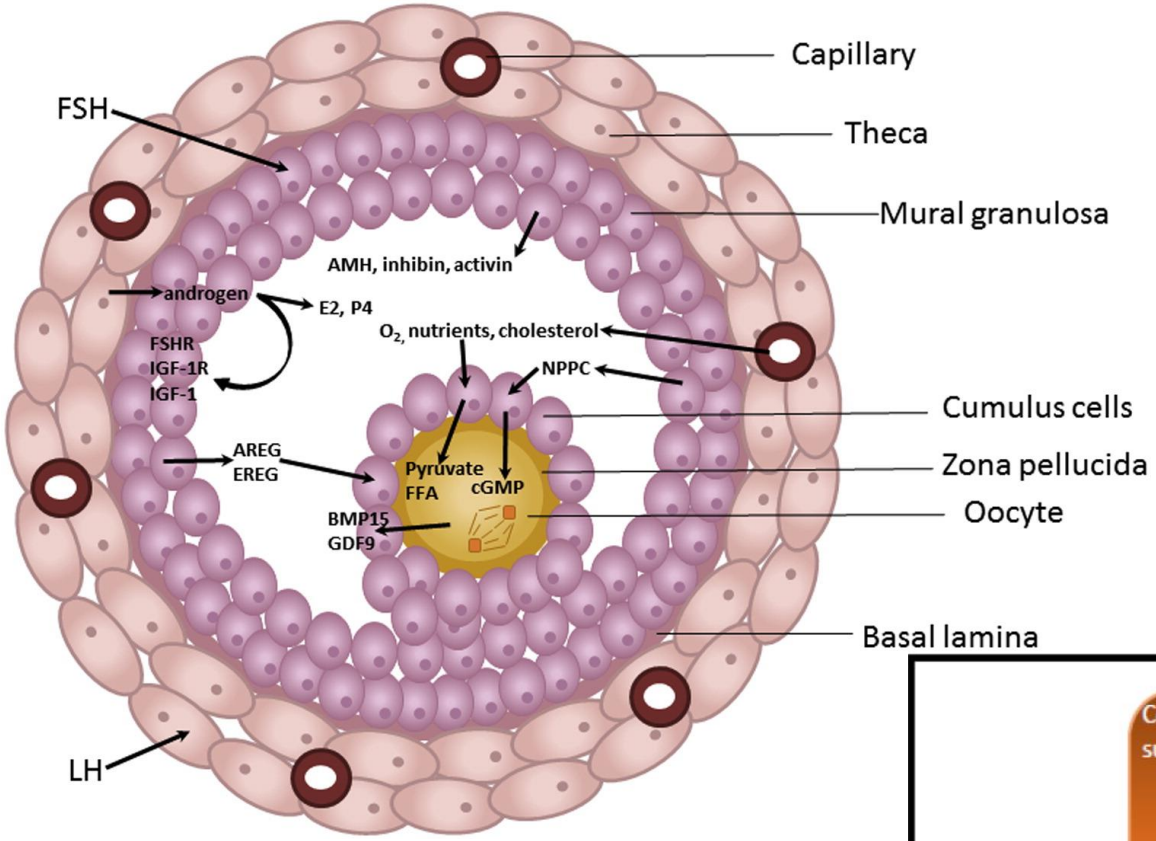
V.le Cavour, Ferrara

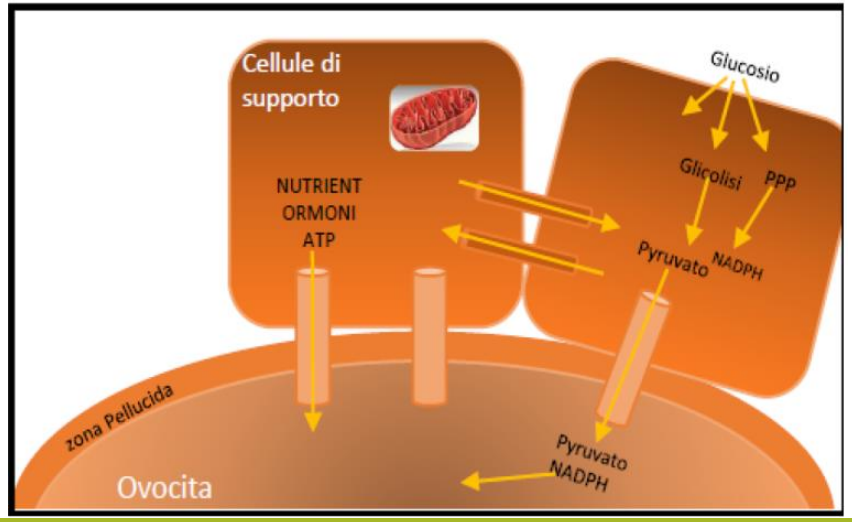
IL RUOLO DELL'ENERGIA MITOCONDRIALE NELLA FISIOLOGIA DEL SISTEMA RIPRODUTTIVO FEMMINILE

Bernard Fioretti

Università degli Studi di Perugia

Ruolo dei Mitocondri nella fisiologia riproduttiva femminile: focus della cellula della granulosa





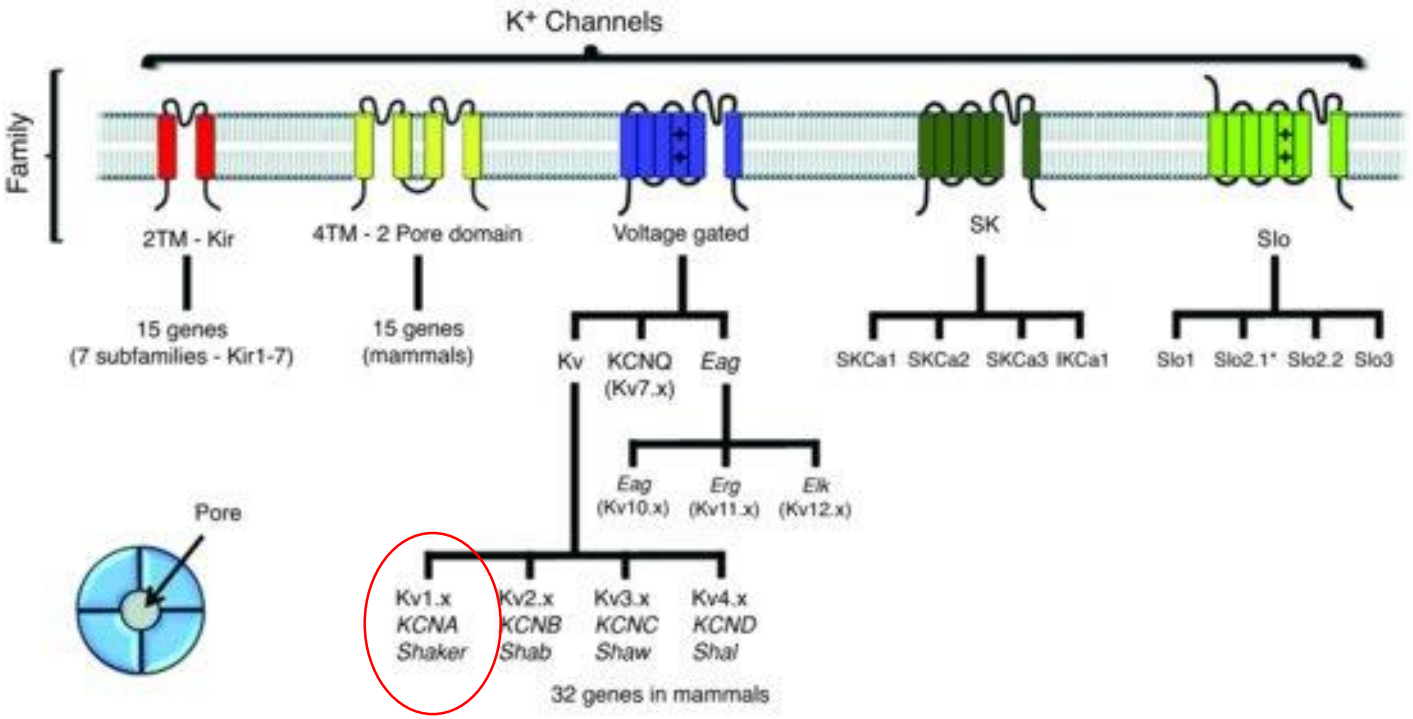
Functional voltage dependent potassium currents in human granulosa cells

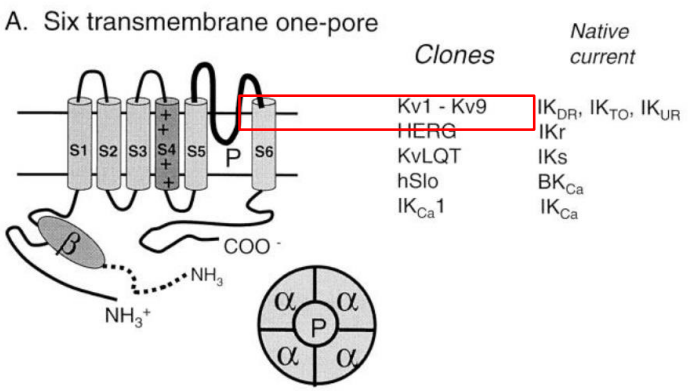
Two principal voltage potassium currents are characterized in mammalian GCs

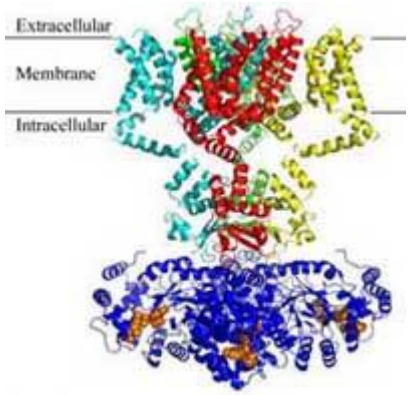
- A slow activating no inactivating current may be ascribed to KCNQ1 with KCNE, defined IKs (Kusaka et al., 1993 and Mattioli, 1993, Mason et al., 2002)
- A fast activating inactivating current, defined IKur (Mattioli et al., 1993 and Manson et al., 2002) with a molecular association to omo and eterotetrameric subunit Kv.1 potassium channel co-expressed with beta regulatory subunit.

Voltage Potassium channels are the principal determinant in the setting of membrane potential and their modulation by FSH and LH are suggested to explain the biological effect promoted by these gonadotropins (Kusaka et al., 1993, Mattioli et al., 1990, 1991, 1993).

Potassium channels



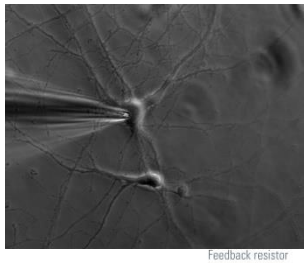


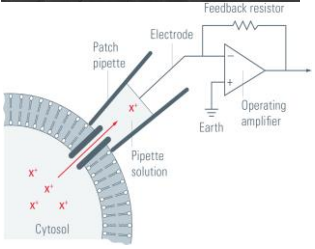


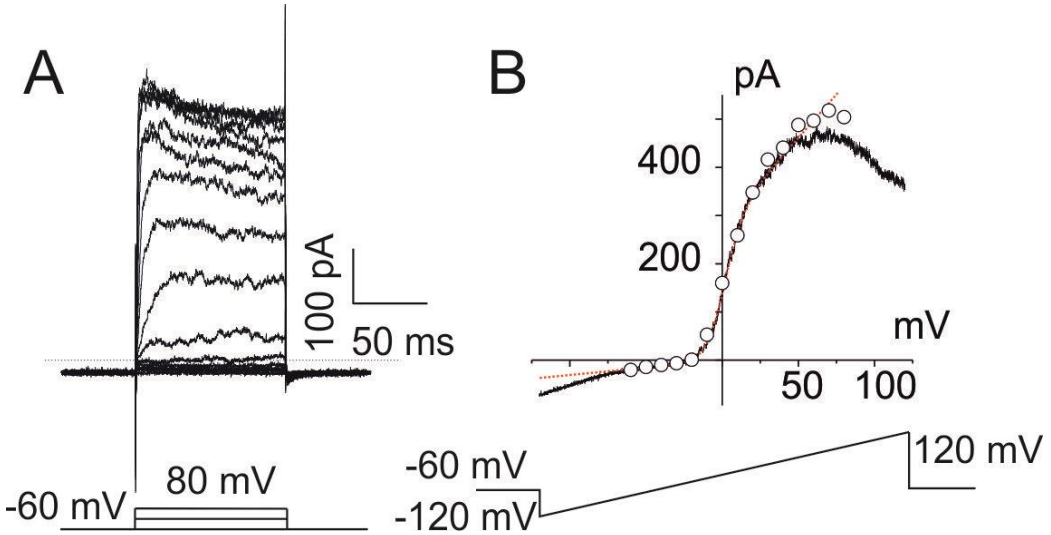
A FAST ACTIVATING INACTIVATING CURRENT (IKUR) IS EXPRESSED IN COV434 HUMAN GRANULOSA CELL LINE

48 h of subculture

Patch-clamp tecnique







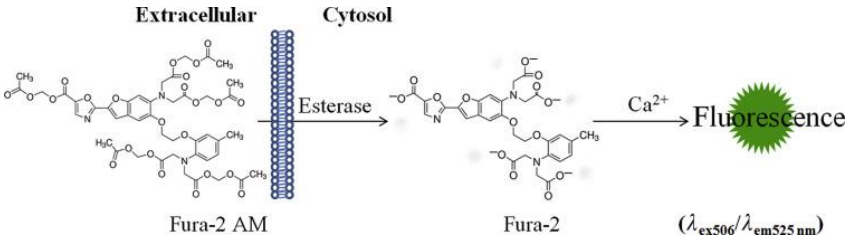
RT-PCR

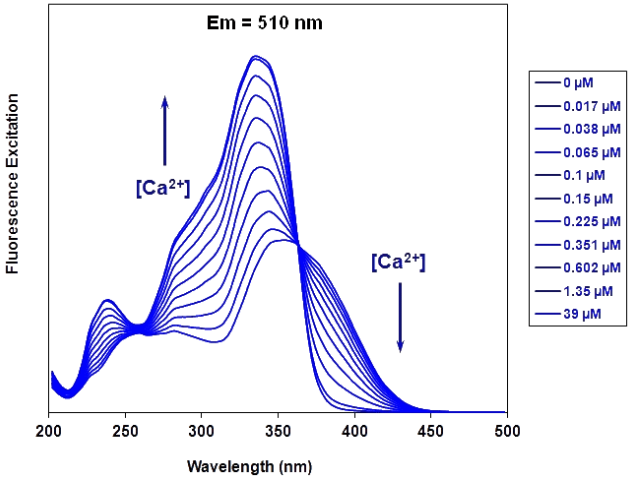
Gene expression of Kv1 subfamily members during time of COV434 subcoltures

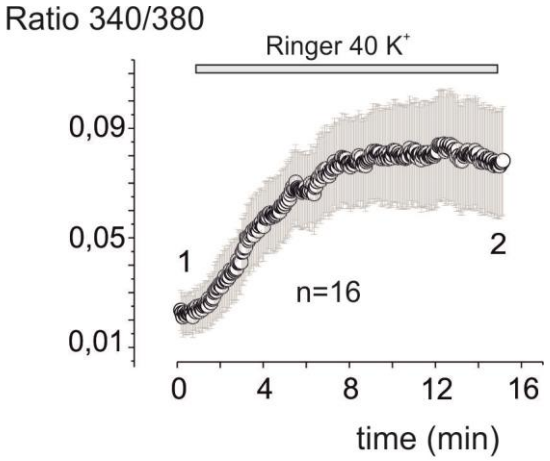
Kv1 subfamily expression table

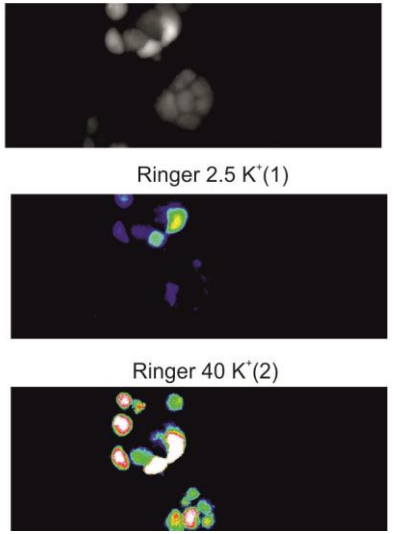
	24h	48h	72h
Kv1.1	-	+	++
Kv1.2	-	-	-
Kv1.3	-	+	-
Kv1.4	-	-	-
Kv1.5	-	-	-
Kv1.6	-	-	-
Kv1.7	-	+	-

DEPOLARIZATION OF MEMBRANE POTENTIAL INCREASE INTRACELLULAR CALCIUM CONCENTRATION IN GRANULOSE CELLS



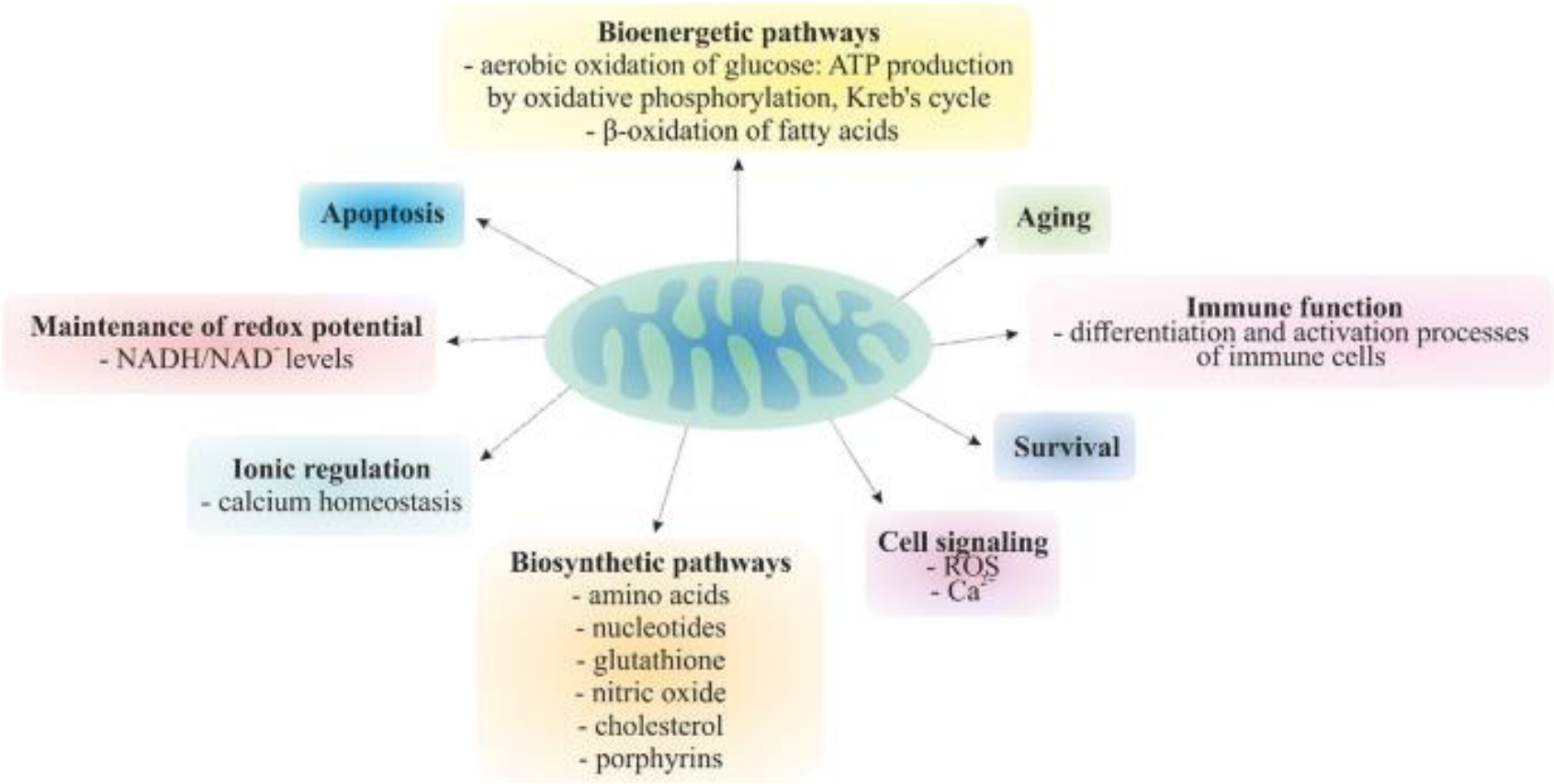






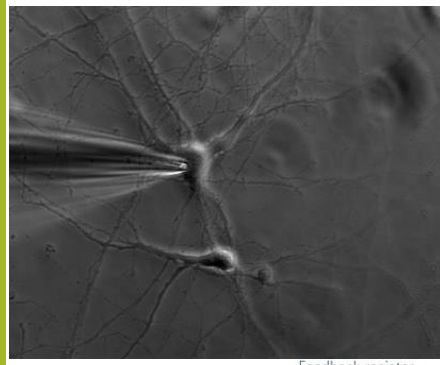
Au HK, Yeh TS, Kao SH, Shih CM, Hsieh RH, Tzeng CR. Calcium-dependent up-regulation of mitochondrial electron transfer chain gene expressions in human luteinized granulosa cells. Fertil Steril. 2005 Oct;84 Suppl 2:1104-8. doi: 10.1016/j.fertnstert.2005.03.072. PMID: 16210000.

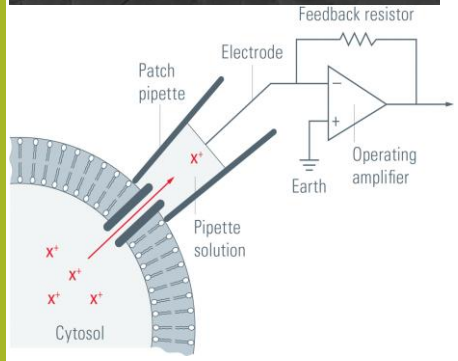
Mitochondrial cellular function



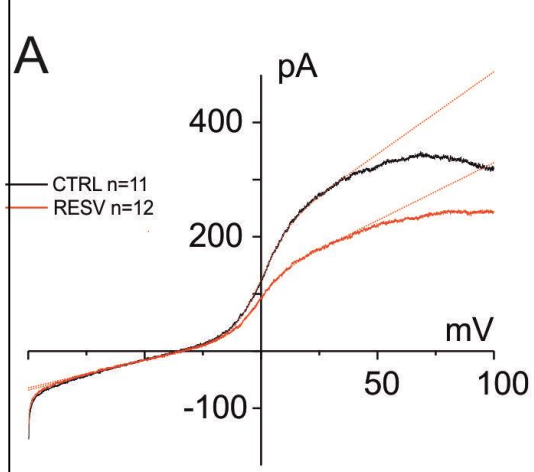
RESVERATROL REDUCE IKUR AND DEPOLARIZED GRANULOSA CELLS

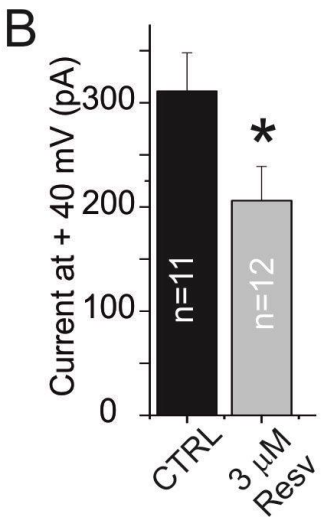
Patch-clamp tecnique





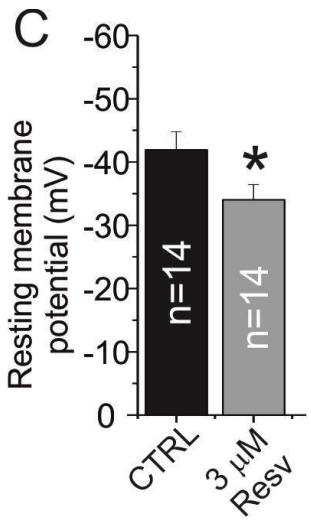
Voltage clamp configuration





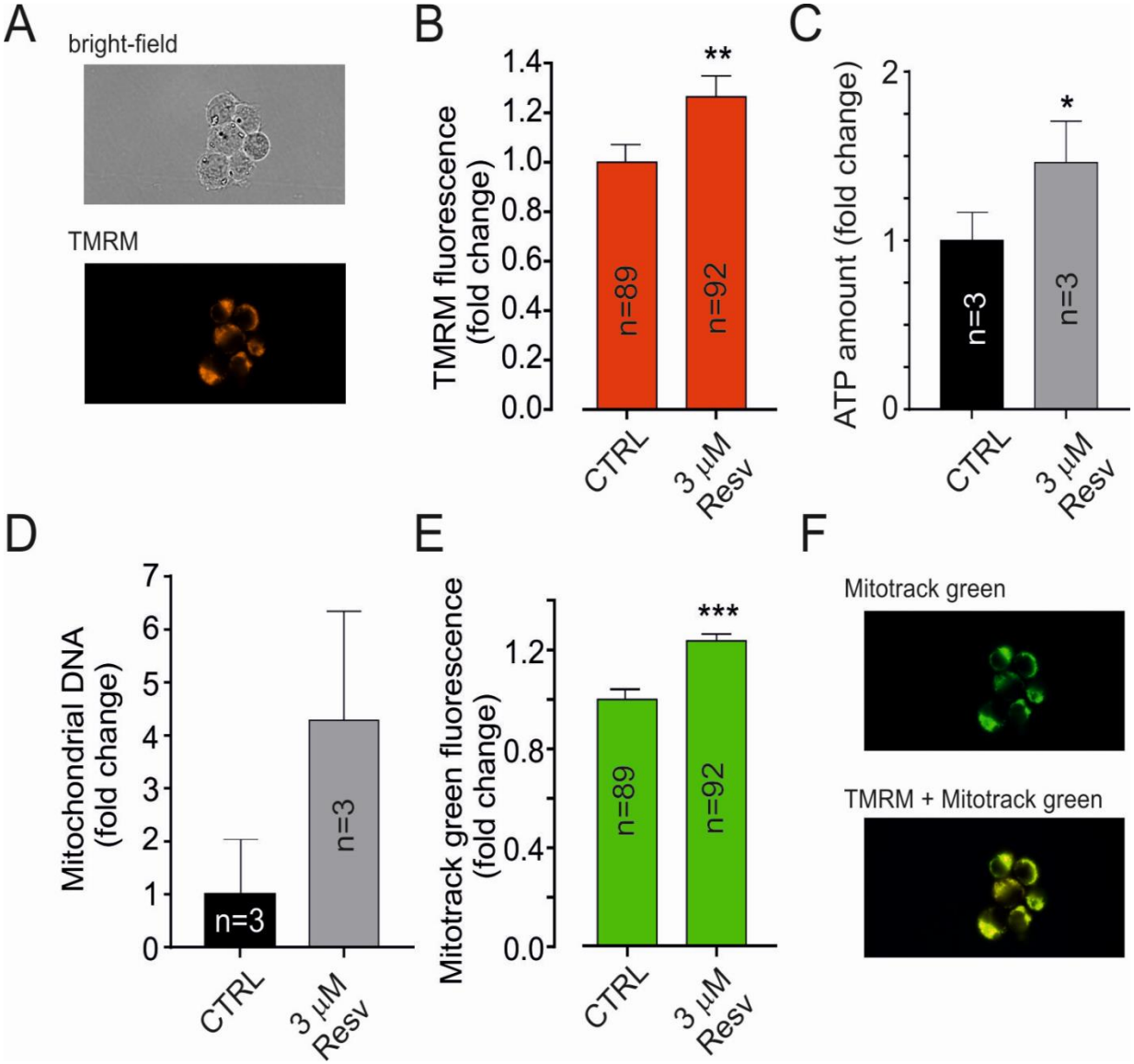
Resveratrol decrease about 30% K currents

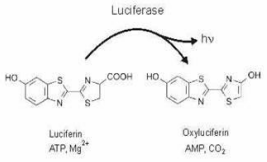
Current clamp configuration



Resveratrol depolarizes about 8 mV resting membrane potential

RESVERATROL INCREASES MITOCHONDRIAL BIOGENESIS AND THE ENERGY STATE IN GRANULOSA CELLS





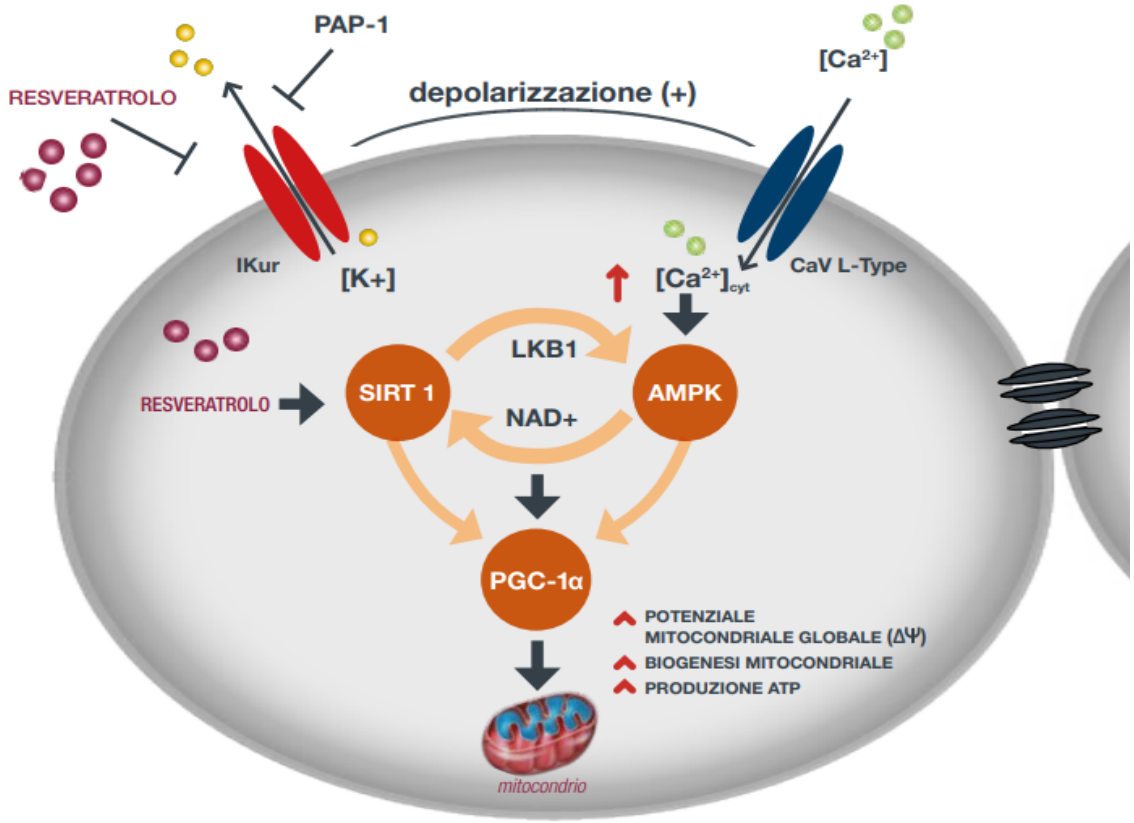
Greater energy related to oxidative phosphorylation

Mitochondrial biogenesis stimulation

Ragonese et al., 2021, Fertility and Sterility,

Cellule della Granulosa fondamentali nel Cross-Talk Energetico

Meccanismo d'Azione



Fertility and Sterility asrm

ORIGINAL ARTICLE | VOLUME 115, ISSUE 4, P1063-1073, APRIL 01, 2021

Resveratrol depolarizes the membrane potential in human granulosa cells and promotes mitochondrial biogenesis

Francesco Ragonese, Ph.D. • Lorenzo Monarca, M.Sc. • Antonella De Luca, Ph.D. • ...
Rossana Giulietta Iannitti, Ph.D. • Lucio Leonardi, M.Sc. • Bernard Fioretti, Ph.D. • Show all authors

Published: January 21, 2021 • DOI: https://doi.org/10.1016/j.fertnstert.2020.08.016 • Check for updates

- Potenziale Mitocondriale
- Biogenesi Mitocondriale
- Produzione di ATP

Adapted from Ragonese F. et al. 2021 Fertility and Sterility

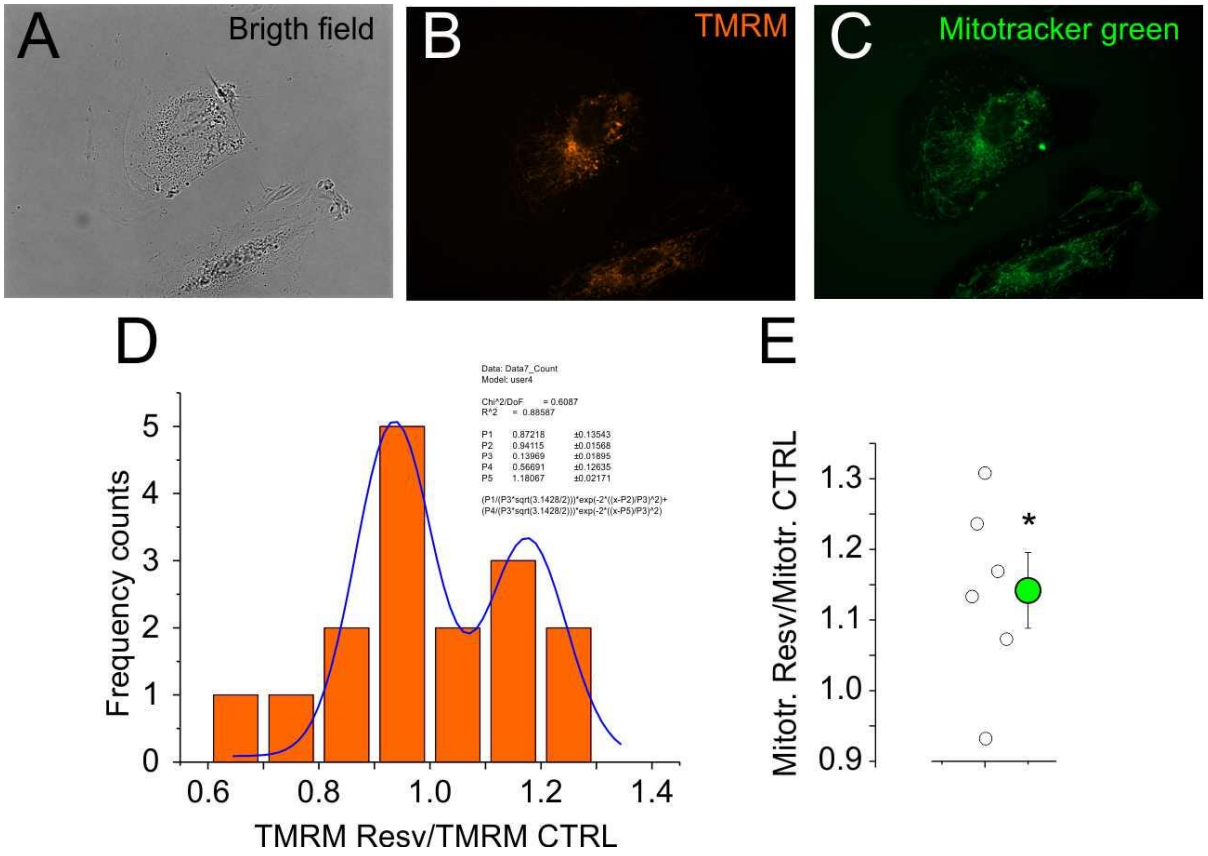
CONCLUSIONI (1)

Granulosa

- Modelli di linee cellulari di granulosa umana (COV434) esprimono Ikur sostenuto a membri Kv.1.1, Kv1,3 e Kv1.7 (dati farmacologici e trascrizionali)
- Ikur modula il potenziale di membrana a riposo e regola i livelli di calcio intracellulare- FSH attiva IKUR (?)
- Ikur è un bersaglio di agenti fisiologici (FSH, LH, ecc) e farmaceutici (resveratrolo) Ragonese et al.2020; Gerli S et.al 2021.

l'attività promossa da nutraceutici modulano l'eccitabilità e la funzionalità delle cellule di supporto –Cellule della Granulosa- e potrebbero spiegare gli effetti positivi di questo nutraceutico rendendolo **un'opzione TERAPEUTICA PER L'INFERTILITÀ**

ATTUALE RICERCA: Cumulus-oocytes complex (COC)



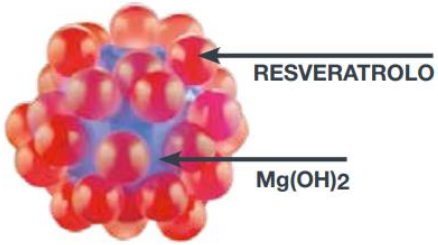
Resveratrolo aumenta il potenziale globale e biogenesi mitocondriale

Fioretti et al. Unpubblished data

REVIFAST: RESVERATROLO PER USO CLINICO

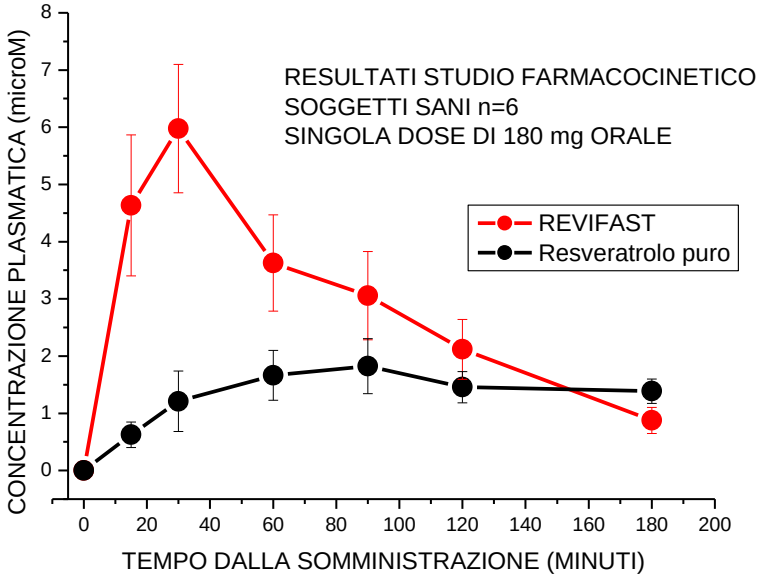
Il Resveratrolo è scarsamente biodisponibile a causa del ridotto assorbimento legato principalmente alla sua bassa solubilità in acqua. L'incremento della biodisponibilità del Resveratrolo è un elemento necessario al fine di sfruttare il reale potenziale farmacologico e salutistico di questo polifenolo.

REVIFAST® è una formulazione brevettata a base di Resveratrolo supportato su una matrice di magnesio d'idrossido



REVIFAST prolunga il tempo di assorbimento del Resveratrolo permettendo di raggiungere concentrazioni plasmatiche maggiori

30 compresse 450 mg



R.G. Iannitti et al. Frontiers in Nutrition 2020

EFFETTI BIOLOGICI PROMOSSI IN VIVO

THE JOURNAL OF MATERNAL-FETAL & NEONATAL MEDICINE
https://doi.org/10.1080/14767058.2021.1958313

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ORIGINAL ARTICLE

Check for updates

Biological and clinical effects of a resveratrol-based multivitamin supplement on intracytoplasmic sperm injection cycles: a single-center, randomized controlled trial

Sandro Gerli[a,b], Chiara Della Morte[a,b], Margherita Ceccobelli[a,b], Monica Mariani[b], Alessandro Favilli[c], Lucio Leonardi[d], Alessandro Lanti[d], Rossana G. Iannitti[d] and Bernard Fioretti[b,e]

[a]Department of Medicine and Surgery, Centre of Perinatal and Reproductive Medicine, University of Perugia, Perugia, Italy; [b]Centre of Assisted Reproductive Technologies, S. Maria della Misericordia Hospital, Perugia, Italy; [c]Department of Obstetrics and Gynecology, AOUI Verona, Verona, Italy; [d]Department of R&D, S&R Farmaceutici S.p.A Bastia Umbra, Perugia, Italy; [e]Department of Chemistry, Biology and Biotechnologies, University of Perugia, Perugia, Italy

ABSTRACT

Background: Resveratrol display's positive effects on follicle growth and development in pre-clinical studies while there is scantly information from clinical trials. The aim of this study was to evaluate the biological and clinical impact of a resveratrol-based multivitamin supplement on intracytoplasmatic sperm injection (ICSI) cycles.

Methods: A randomized, single-center controlled trial conducted at the University Center of Assisted Reproductive Technologies involving 101 women infertile women undergoing ICSI cycles was conducted. A pretreatment with a daily resveratrol based nutraceutical was administered to the Study Group; Control Group received folic acid. The primary outcomes were the number of developed mature follicles (>16 mm), total oocytes and MII oocytes recovered, the fertilization rate and the number of cleavage embryos/blastocysts obtained. Secondary endpoints were the duration and dosage of gonadotropins, the number of embryos for transfer, implantation, biochemical, clinical pregnancy rates, live birth and miscarriage rates.

Results: A significantly higher number of oocytes and MII oocytes were retrieved in the Study Group than in Control Group ($p = .03$ and $p = .04$, respectively). A higher fertilization rate ($p = .004$), more cleavage embryos/patient ($p = .01$), blastocytes/patients ($p = .01$) and cryopreserved embryos ($p = .03$) were obtained in the Study Group. No significant differences in biochemical or clinical pregnancy, live birth, and miscarriage rates were revealed, but a trend to a higher live birth rate was revealed in the Study Group.

Conclusions: A 3 months period of dietary supplementation with a resveratrol-based multivitamin nutraceutical leads to better biological effects on ICSI cycles.

Trial registration number: ClinicalTrials.gov registration identifier: NCT04386499

ARTICLE HISTORY
Received 15 July 2021
Accepted 18 July 2021

KEYWORDS
Resveratrol; intracytoplasmic sperm injection; assisted reproductive technologies; embryo; oocyte

Table 2. Gonadotropins, follicles and oocytes in the Control and Study groups.

	Control Group ($n = 50$)	Study Group ($n = 40$)	p
Days of stimulation	10.5 ± 0.2	11.2 ± 0.3	.06
Gonadotropins (IU)	2693.4 ± 141.4	2480.6 ± 131.6	.27
Estradiol levels at the day of hCG (pg/mL)	2790.3 ± 567.3	2272.8 ± 378.6	.47
Number of follicles (>16 mm)	9.4 ± 0.6	10.9 ± 0.8	.13
Oocytes retrieved	7.1 ± 0.4	8.7 ± 0.7	.03
Ratio follicles/ oocytes retrieved	0.77 ± 0.02	0.82 ± 0.03	.24
MII oocytes	4.9 ± 0.4	6.4 ± 0.6	.04

S.Gerli et al. J Matern Fetal Neonatal Med. 2021

EFFETTI BIOLOGICI PROMOSSI IN VIVO

Table 3. Embryos and pregnancies in the Control and Study groups.

	Control Group ($n = 50$)	Study Group ($n = 40$)	p
Fertilization rate	64.6% (210/325)	75.4% (230/305)	.004
Fertilized oocytes/patient	4.4 + 0.4	5.7 + 0.4	.01
Cleavage rate	94.1% (208/210)	96.5% (222/230)	.08
Cleavage embryos/patient	4.2 ± 0.4	5.7 ± 0.4	.01
Blastocysts/patient	2.3 ± 0.3 (89/38)	3.5 ± 0.3 (104/30)	.01
Transferred embryos/patient	1.8 ± 0.1	1.9 ± 0.1	.3
Embryos transferred day 3	39% (31/79)	27% (20/74)	.11
Embryos transferred day 5	61% (48/79)	73% (54/74)	.11
Cryopreserved embryos/patient	1.1 ± 0.21	1.5 ± 0.3	.03
Implantation rate	25.3% (20/79)	24.3% (18/74)	.96
Biochemical pregnancy rate (BPR)	42.8% (18/42)	43.2% (16/37)	.84
BPR (transfer day 3)	40.0% (6/15)	44.4% (4/9)	.83
BPR (transfer day 5)	44.4% (12/27)	42.9% (12/28)	.91
Clinical pregnancy rate (CPR)	40.5% (17/42)	37.8% (14/37)	.99
Clinical pregnancy rate (CPR)[a]	34.0% (17/50)	28.6% (14/49)	.56
CPR (transfer day 3)	33.3% (5/15)	33.3% (3/9)	.99
CPR (transfer day 5)	44.4% (12/27)	39.3% (11/28)	.70
Live birth rate (LPR)	30.9 % (13/42)	35.1 % (13/37)	.87
Live birth rate (LBR)[a]	26.0 % (13/50)	26.5 % (13/49)	.95
LBR (transfer day 3)	13.3% (2/15)	22.2% (2/9)	.57
LBR (transfer day 5)	40.7% (11/27)	39.3% (11/28)	.91
Delayed transfer rate	10.6% (5/47)	2.6% (1/38)	.31
Delayed transfer rate[a]	10.0% (5/50)	2.0% (1/49)	.1
Miscarriage rate	23.5% (4/17)	21.4% (3/14)	.77
Miscarriage rate[a]	8.0% (4/50)	6.1% (3/49)	.71

Values are expressed as mean ± SE or percentages (numbers in parenthesis).
[a]The statistical analysis was made on the intent-to-treat (ITT) population (Ctrl = 50 and Study Group = 49, for details see Figure 1).

S.Gerli et al. J Matern Fetal Neonatal Med. 2021

Conclusions & Future directions

- **Human granulosa cell line models (COV434) expressed Ikur sustained to Kv.1.1, Kv1,3 and Kv1.7 members (pharmacological and transcriptional data)**
- **Ikur set the resting membrane potential and regulates intracellular calcium levels**
- **Ikur is a target of physiological (FSH, LH, etc) and pharmaceutical agents(Resveratrol)**
- **Resveratrol-based Nutraceutica represent a clinical opportunity to promote follicologenesis**

The functional coupling between membrane potential, intracellular calcium and mitochondrial biogenesis represents the future direction of our research and clinical application in women and male fertility

Acknowledgment

COLLABORATORS

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