PROGETTO PATROCINATO DA

SIA Società Italiana di Andrologia

SOCIETÀ ITALIANA DI ANDROLOGIA

PROGETTO PATROCINATO DA

SIU

SOCIETÀ ITALIANA DI UROLOGIA

FONDATA NEL 1903

Società Italiana di Riproduzione Umana

EcoFoodFertility

ambiente, alimentazione, fertilità

Valutazione dell'Impatto Ambientale ed Alimentare sulla Funzione Riproduttiva Maschile

COORDINATORE

Dott. Luigi MONTANO UroAndrologo - ASL SALERNO

Ministero della Salute

INTERNATIONAL SOCIETY OF DOCTORS FOR ENVIRONMENT

ISDE

ASL Salerno

Azienda Sanitaria Locale Salerno

Un modello di intervento per la prevenzione dell'infertilità in adolescenti sani residenti in aree a forte impatto ambientale

X Giornata Italiana Medici per l'Ambiente

Arezzo 29-30 settembre 2017

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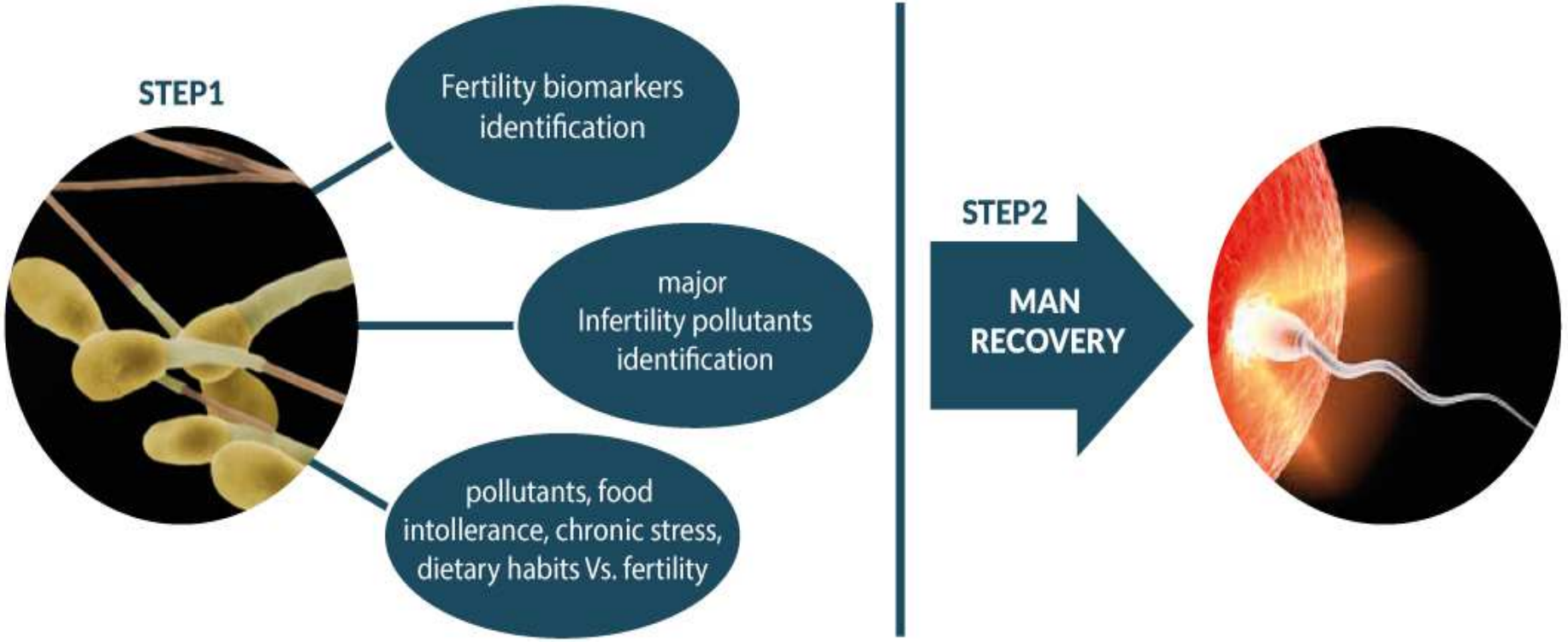

It is a human biomonitoring study with multidisciplinary approaches involving, environment, life-style and diet, using the qualitative and quantitative alterations of human semen, as a key to understand both the level of environmental quality and its long term modifications to set out health risks for populations in relation with their living environment as well as diet and lifestyle.

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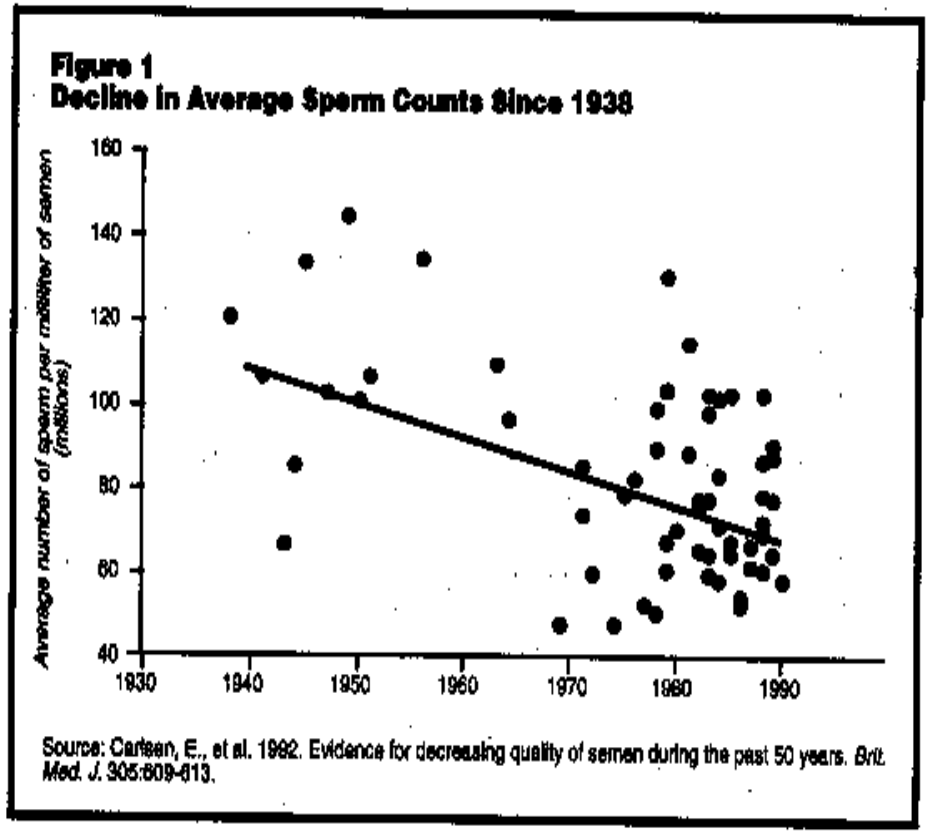
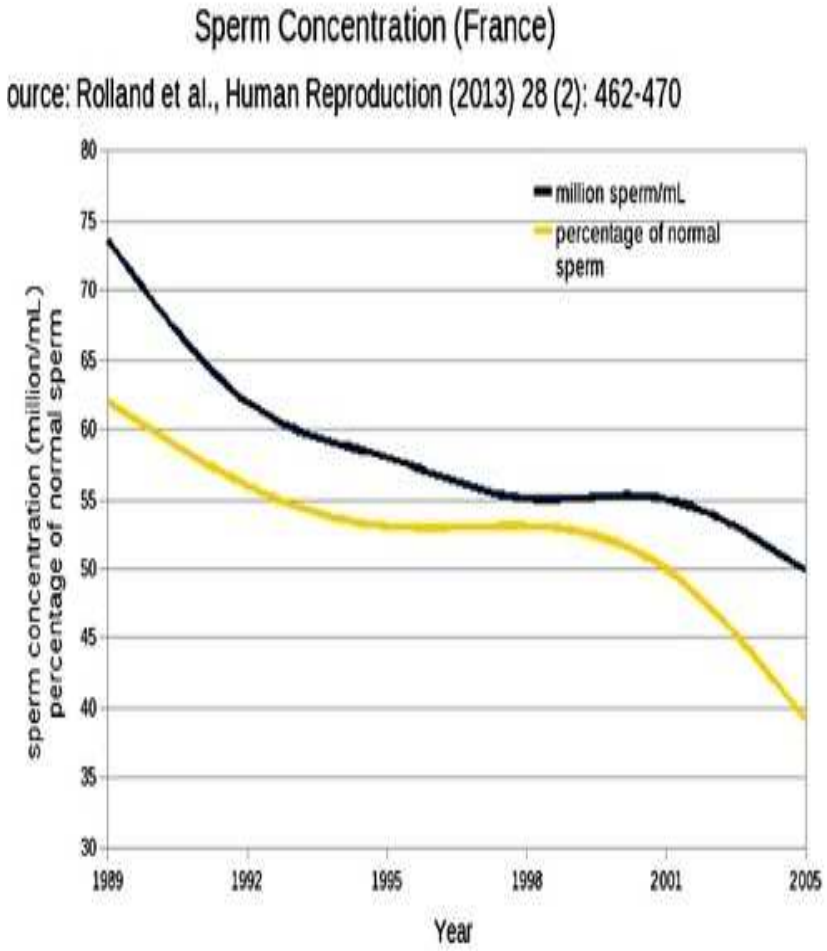
The performance of the quality and quantity of the sperm in the last 70 years

1992 (Carlsen et al., 1992)



Danish researchers combined the results of 61 studies from around the world to show that average sperm counts had dropped about 50 percent in the last 50 years.

2013 (Le Moal J et al. 2013)



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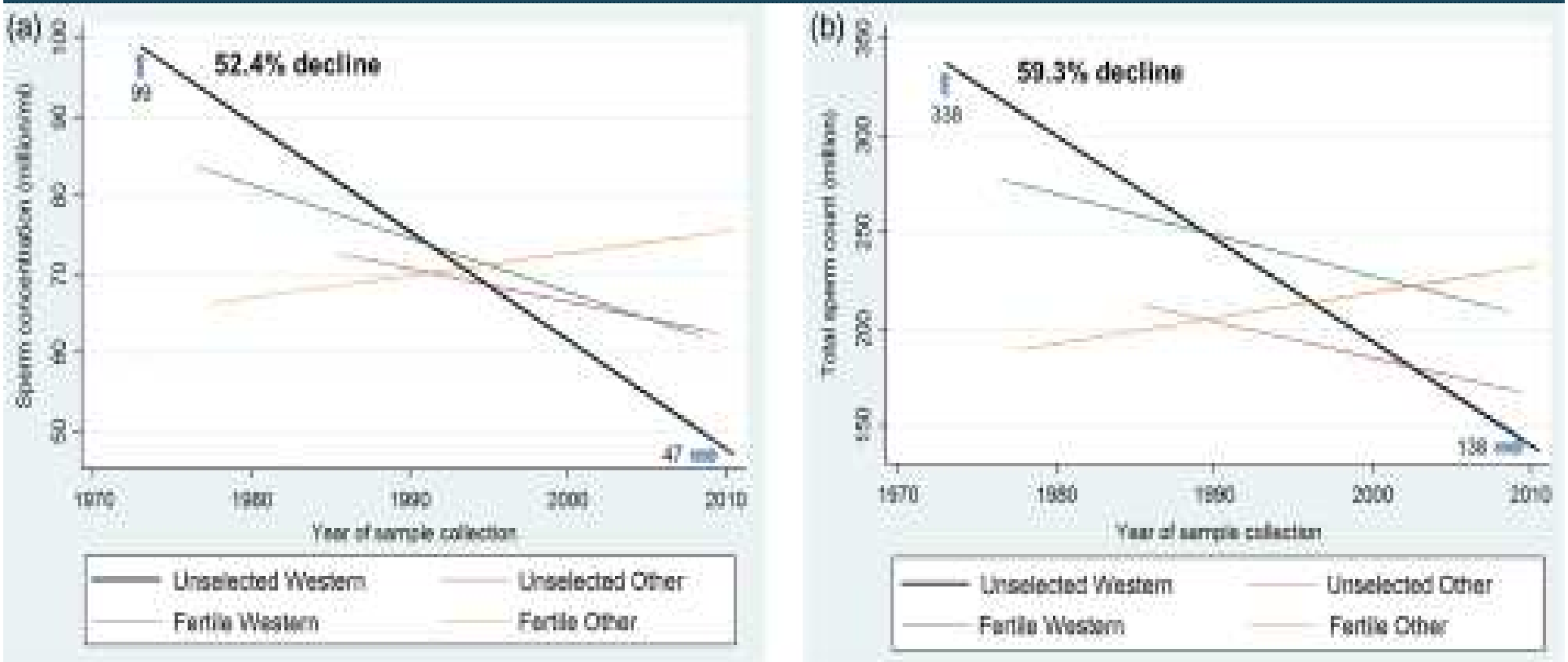
Levine et al. (2017)



The incidence of male infertility is increasing in industrial countries from 7% - 8% in 1960 to 20% - 35% in 1990, and persists to augment incessantly.

This dramatic growth of infertility is becoming a top priority for the public health

These decline lead us to consider human semen as the most faithful mirror of lifestyle and environmental changes in the last 70 years in western countries

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WHY HUMAN SEMEN?
Elective biomarker of environmental exposure

1) **The decline of human sperm is already an epidemiological information on high environmental sensitivity, considering also differences in areas within the same country or even in the same region that support the fact that environmental factors, present in some areas but not in others, may be responsible for the decline in semen quality** [Nordkap L. et al 2012, Zhou N et al. 2014, Bergamo et al. 2016]

2) **Epidemiological studies in exposed individuals for professional reasons and in the inhabitants of areas contaminated by nearby settlements, show: Reduction of sperm motility, concentration, morphology, DNA sperm damage, repeated abortions and genetic and epigenetic damage**

3) **Many Toxicology studies in animal models show how some of the major environmental organic and inorganic contaminants reduce seminal quality**

4) **In fact, the pollution impact, generally to several pollutants simultaneously influence both quantity and quality of gametes and the mechanisms put forward in the literature, that probably working in combination are: genetic and epigenetic alterations, hormonal imbalance, oxidative stress induction** [Carré J et al. 2017]

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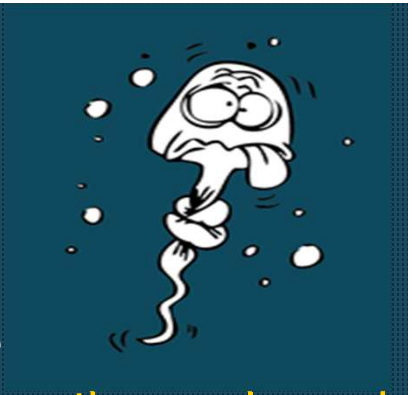

WHY HUMAN SEMEN?
Elective biomarker of environmental exposure

- Spermatogenesis unlike oogenesis from puberty onward is continuously and therefore more easily exposed to insults in his stages of continuous replication and so male germline accumulates mutations faster than female one **[Justin P et al. 2007, Ségurel L et al. 2014]**

- Sperm cells are more susceptible than eggs to the effects of oxidative damage, because they lack significant antioxidant protection because of reductive cytoplasmic space for an appropriate armoury of defensive enzymes and significant amounts of polyunsaturated fatty acids. **[Aitken RJ et al. 2016]**

- Furthermore in semen simultaneously is possible measure environmental contaminants and in vivo effects on sperm cells, which are readily available, with features sensitive to environmental pollutants such as motility, morphology and the integrity of the DNA strand.

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HEALTH MARKER

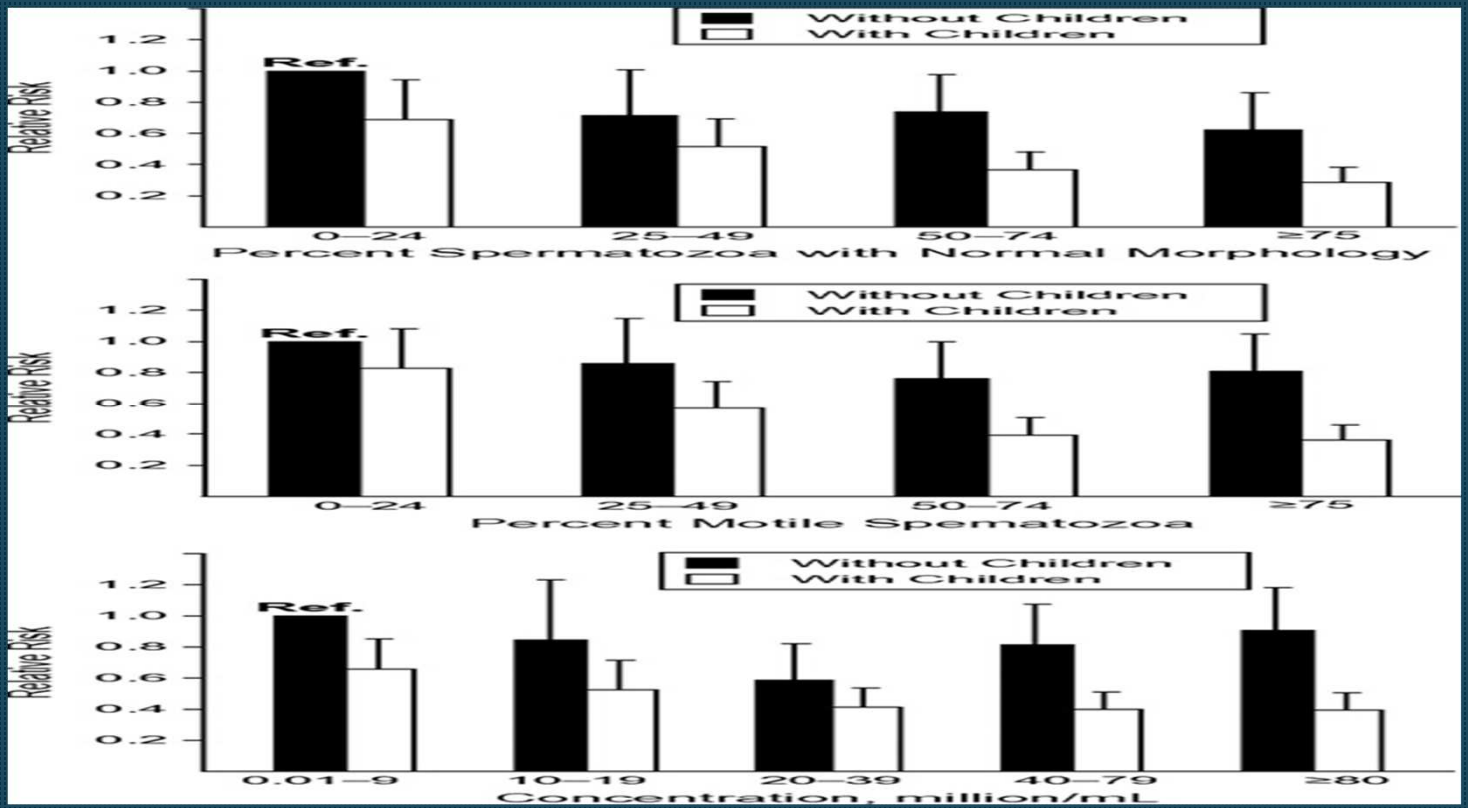
2009 Jensen TK et al. Good semen quality and life expectancy: a cohort study of 43,277 men. Am J Epidemiol ;170: 559–565

2014 Eisenberg ML et al. Semen quality, infertility and mortality in the USA. Hum Reprod., 29:1567-74

2015 Eisenberg MLelationship between semen production and medical comorbidity. Fertil Steril. 103:66-71.

2017 Latif T et al. Semen quality is a predictor of subsequent morbidity. A Danish cohort study of 4,712 men with long-term follow-up. Am J Epidemiol. 2017 May 11. doi: 10.1093/aje/kwx067

Relative risk of death according to fertility status and percent of sperm aged 50 years in 1980.



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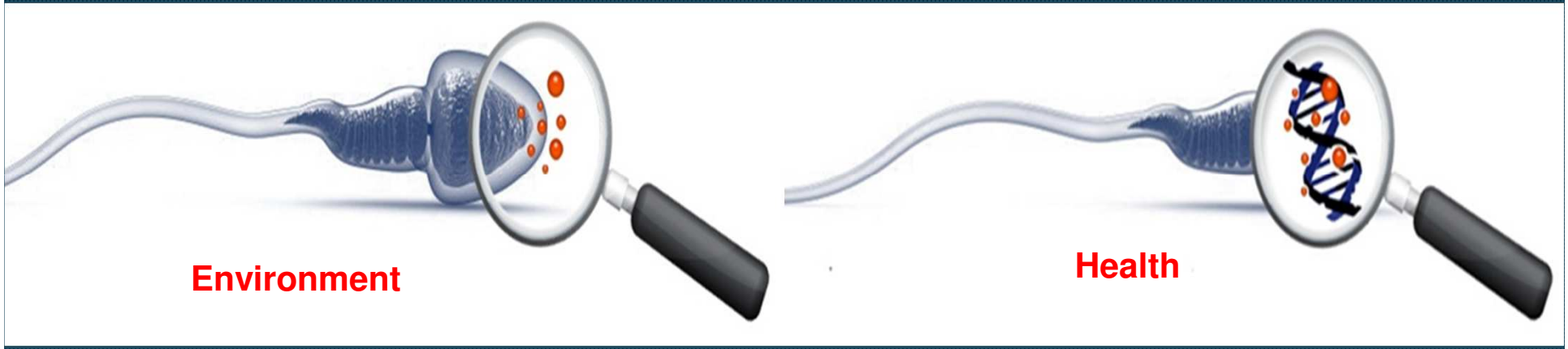
These epidemiological and toxicological data show how the male reproductive system is particularly sensitive to exogenous (pollution) and endogenous stress, therefore with preventive and/or mitigation aim of environmental effects on human health of EcoFoodFertility Project the MALE REPRODUCTIVE SYSTEM seems ideal to seize the first signs of harmful effects on human health arising from the pollution as a

SENTINEL ORGAN and Human Semen an ideal "KEY" of the relationship between the Environment and Health



THE DUAL FUNCTION OF HUMAN SEMEN

"Environmental and Health Marker"

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AIMS

i) Developing a better understanding of the effects of environmental pollutants on human semen parameters, evaluating qualitative profiles and the bio-accumulation, in homogeneus subjects living in areas with different environmental pressures;

ii) Qualifying human semen as an early and sensitive Environment and Health Marker;

iii) Proposing "*Human Seminal Model*" for early detection and prevention of environmental health risks, useful in innovative programs on health surveillance;

iv) Identifying lifestyle and especially Dietary approaches with anti inflammatory, antioxidant and detoxifying properties may be effective in mitigating and reducing environment impact (pollutant bioaccumulation) on human health improving human semen quality in men living in polluted areas;

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WHY?

NEED FOR TRUTH

Health Status of Population of «Land of Fires» (Campania Region in Southern Italy)

"*Land of Fires*" is an area of Southern Italy illfamed for multiplicity sources of pollution (illegal disposal of urban, toxic and industrial wastes, dumping practices, traffic, intensive agriculture), widespread on high territorial extension with 2,5 million inhabitants. This area probably has no equal in the world for exposome studies

Verifying biohazard indexes through checking the status of contaminants bioaccumulation and biomarkers of effect and susceptibility in homogeneous samples of population living in areas with different environmental pressure index and proposing innovative preventive programm to safeguard public health

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Participant No	Participant institutions involved in first call of Horizon EU Programm	Acronym	Country
1 Scientific coordinator	National Research Council: ISA – IFC - ISTM ISTEC – ISPAAM - IMT	CNR	ITALY
2 partner	University of Naples – Dept. of Environment and Earth Science	UNINA	ITALY
3 partner	University of Heidelberg (Uro-Andrology Clinik of Heilbronn)	SLK	GERMANY
4 partner	University of Athens - Medical School – Dept. of Ecotoxicology	UOA	GREECE
5 partner	Consorci Sanitari de Terrassa	CST	SPAIN
6 partner	CatLab	CATLAB	SPAIN
7 partner	University of Budapest (National Institute of Andrology)	SE	HUNGARY
8 partner	Veterinary Research Institute – Genetics and reproduction	VRI	CZECH REPUBLIC
9 partner	Institute of Experimental Medicine - Academy of Sciences of the Czech Republic	IEM	CZECH REPUBLIC
10 Partner	ISDE – Intern. Soc. Doctor for	IDEA	IRELAND

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European Commission

S&D

Group of the Progressive Alliance of
Socialist & Democrats
in the European Parliament

6 DECEMBRE 2016
16:00 - 19:00 - Room ASP1H3
European Parliament - Bruxelles

Hosted by **Nicola Caputo MEP**

fratellicapone.it - 0818957866

Health risk prevention in EU areas characterized by High Environmental Pressure

Speakers:

16:10
Luigi Montano
Urologist, Coordinator of EcoFoodFertility project, ASL Salerno - Italy
Human semen as an early and reliable tool of environmental impact assessment on human health, useful for innovative prevention programs and health surveillance in risk areas

16:25
Aleksandra Fucic
Sci adviser, Institute for Medical Research and Occupational Health - Croatia
Biomonitoring of newborns and children associated with exposure to genotoxic agents

16:35
Elpida Niki Emmanouil-Nikoloussi
Histology-Embryology - European University Cyprus
Prevention of birth defects in contaminated areas

16:45
Jean Pierre Bourguignon
Neuroendocrinology Unit, GIGA Neurosciences, Univ. Liège - Belgium
Neurodevelopmental disruption from prenatal life to adolescence

17:00
Alberto Mantovani
Senior toxicologist, Istituto Superiore di Sanità, Roma - Italy
From AOPs to biomarkers: the contribution of toxicological data sets to health risk prevention in children

17:10
Stefania Ubaldi
President of the European Life Style Medicine Organization, Geneve - Switzerland
Lifestyle as a tool to modulate the environmental impact on health

17:20
Maurizio Dattilo
Parthenogen, Lugano - Switzerland
Epigenetic-environment interaction and the role of diet

17:30
Josaine Masson
Policy Officer at the European Commission
Soil protection and sustainable land use

17:40
Michel Pletchette
Adviser at the European Commission on health science

17:50
Hubert Deluyker
Scientific Adviser to the EFSA Executive Director
Approaches for linking scientific developments with risk assessment methodologies

18:00
Verstraete Frans
DG Health and Food Safety
Environmental contaminants in food

18:10
Ann Uutsalu
Programme Officer - DG Research

18:20
Question & Answers

Partner of EcoFoodFertility project
Jan Ludwicki *(Poland)*
Aleksandros Kopa *(Hungary)*
Jri Rubens *(Rep. Ceck)*
Radim J. Sram *(Rep. Ceck)*
Jesus Fernandez Castiñeiras *(Spain)*

Interpretation in IT-EN

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Countries involved in first Call of Horizon EU Programm
Italy, Germany, Czech Rep., Spain, Greece,
To involve in the future in 2018 : Croatia, Belgium, Poland

RECRUITING SUBJECTS

18-40 healthy men, no smokers, no drinkers , no professional exposed, no chronic deseases

Medical examination — **Withdraw** — **Food and LifeStyle Questionnaire**

SEMEN	microbiome	BLOOD	Female Protocol: Follicular fluid on going to….

Semen analysis + DNA integrity (by SCD), sperm aneuploidy study, epigenetic evaluation on sperms
Heavy metals , PAHs , dioxins , PCBs , nanoparticles , Bisphenols , Phthalates , Parabens , Pfoas,Pesticides.
RedOx status, lipidomc status, metabolomics, proteomics, antioxidant enzymes,

Blood analysis and hormone dosage Cytogenetic analysis. Genetic analysis with study polymorphisms of genes involved in the metabolic detoxification mechanisms and DNA repair to the definition of individual susceptibility - Heavy metals , PAHs , PCBs Dioxins , nanoparticles , bisphenols , Phthalates , Parabens , Pesticides,. Rating redOx state, epigenetics and other omic analysis, microbioma

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Men recovery: dietary approach

A Randomized Clinical Trial (RCT) will be carried out on three groups to evaluate the effects of diet for three months on exposure and effect semen biomarkers

- Organic vegetarian diet
- Vegetarian diet
- Normal diet

AIM

Verifying if different dietary approaches may be effective in modifying pollutants bioaccumulation and effect biomarkers (semen parameters, DFI, sperm telomere lenght, semen redOx status, sperm epigenetic, lipidomic status, metabolomic, proteomic, antioxidant enzymes)

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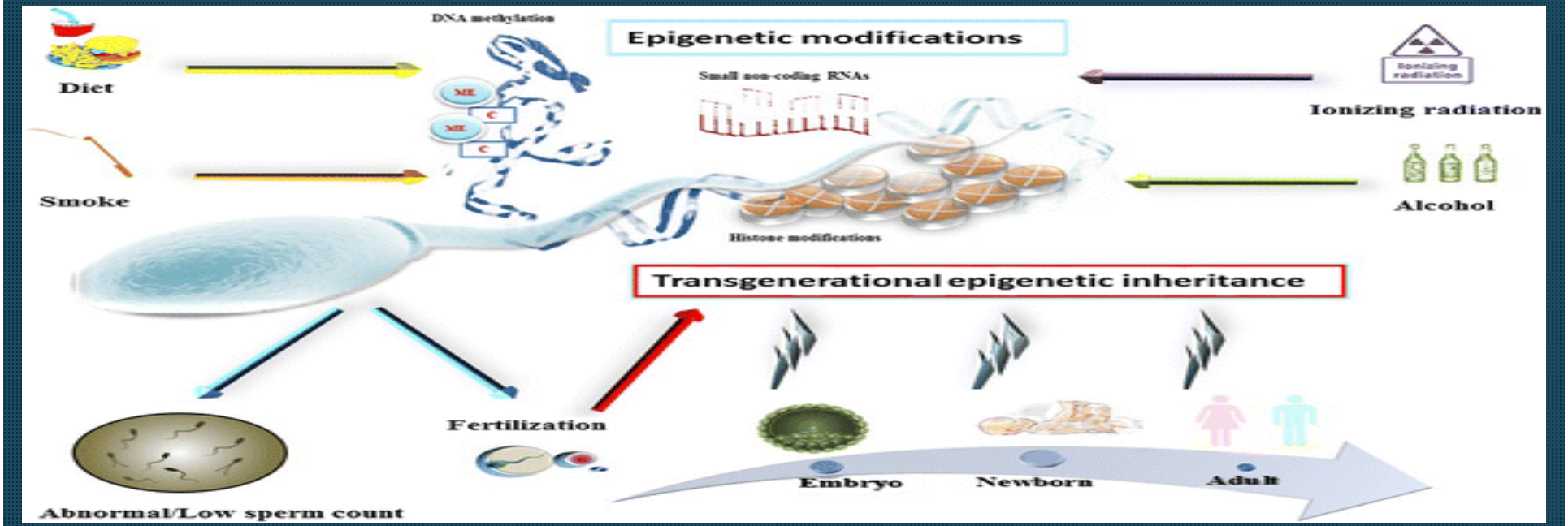
FUTURE GENERATIONS?

Epigenetics and male reproduction: the consequences of paternal lifestyle on fertility, embryo development, and children lifetime health. Clinical Epigenetics . **November 2015**
Stuppia , Franzago, Ballerini, Gatta, Antonucci

Environmental pollutants: genetic damage and epigenetic changes in male germ cells Environmental Science Pollution., September **2016** (23:23339–23348)
Vecoli, Montano, Andreassi

Fertility treatments and pediatric neoplasm of the offspring: results of a population-based cohort with a median follow-up of 10 years. Obstetrics and Gynaecology , January **2017**
Wainstock, Walfisch, Vardi, segal, Harlev, Sergienko, Landau, Sheiner



Transgenerational Effect

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RETE NAZIONALE ECOFOODFERTILITY

Rete per la Salute Ambientale e Riproduttiva (RE.S.A.R.)

Un'Alleanza per la Salvaguardia della Fertilità nelle Aree a Rischio

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ECOFOODFERTILITY EDUCATION

Verso una Rete Internazionale Educativa per la Salute Ambientale e Riproduttiva

R.I.E.S.A.R

PROGETTO PER LE SCUOLE

ECOFOOD FOR LIFE

AMBIENTE – SCELTE ALIMENTARI – STILI DI VITA – TERRITORIO – CITTADINANZA ATTIVA

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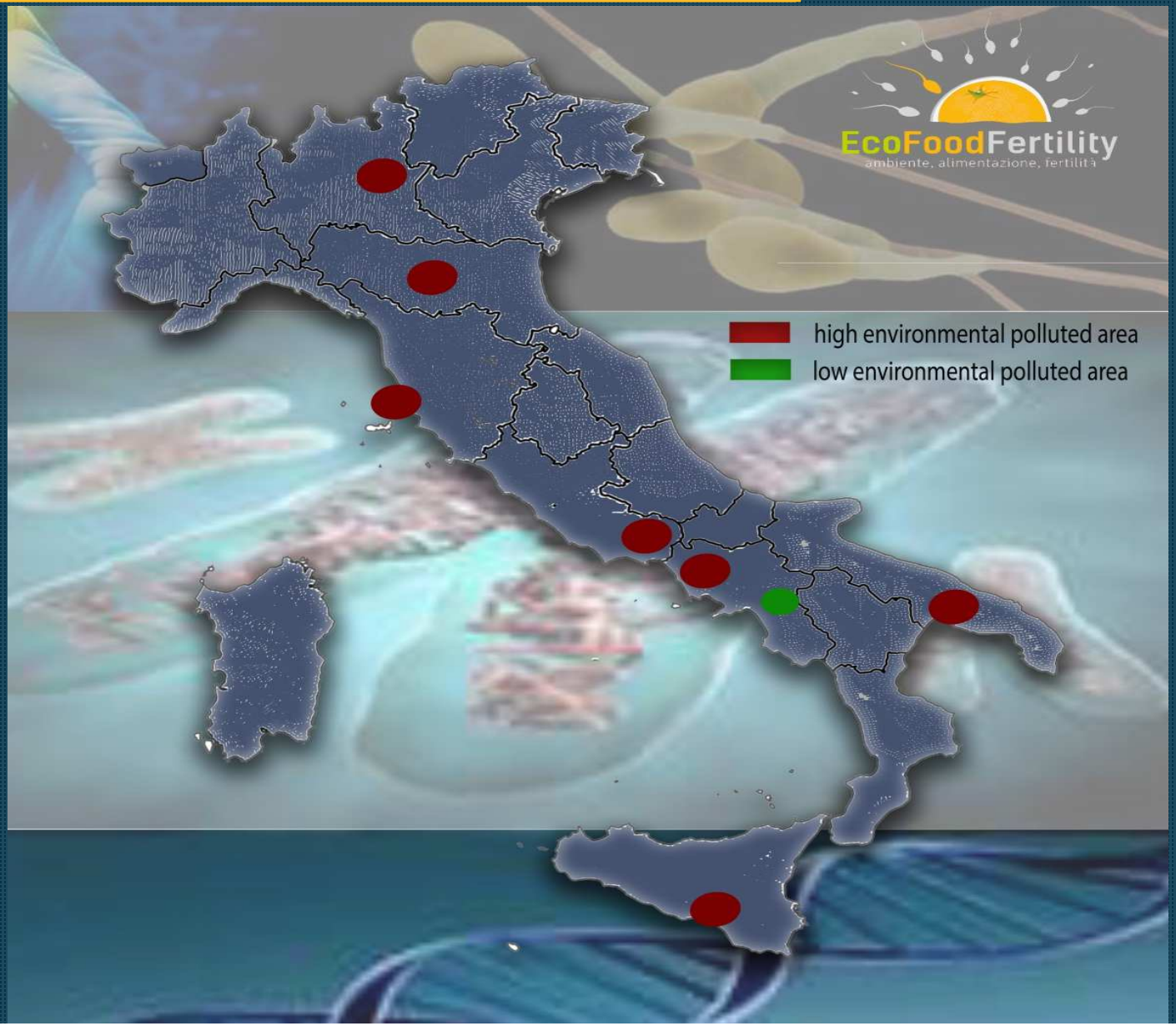
National Project



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PROGETTO NAZIONALE

Regioni	Area ad ALTO impatto ambientale	Area a BASSO impatto ambientale
Abruzzo	Bussi sul Tirino (PE)	Guardiagrele (CH) - Area Parco della Maiella
Campania	Area Nord della Provincia di Napoli	Area dell'Alto-Medio Sele (SA)
Emilia-Romagna	Comprensorio ceramico Sassuolo, Fiorano, Maranello (MO)	Area Marina di Cervia (RA)
Lazio	Valle del Sacco (FR)	Area di Ladispoli (RM)
Lombardia	Sito di interesse nazionale Brescia-Caffaro	Aree non industriali della provincia di Brescia
Puglia	Taranto	Costa salentina della provincia di Lecce
Sicilia	Gela (CL)	Area delle Madonie (PA)
Toscana	Piombino (LI)	Area San Giuliano Terme, Calci, Cascina (PI)
Veneto	Monselice (PD)	Pieve di Cadore (BL)

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Partner aggiunti per la Nuova Proposta Progettuale in EUROPA (2018)

14. Istituto Nazionale di Salute Pubblica – Dipartimento di Tossicologia e Valutazione del rischio – Varsavia, Polonia (Prof. Jan K. Ludwicki)
15. Università di Siviglia- Dipartimento di Urologia – SPAGNA (Prof. Jesus Castineiras Fernandez)
16. Institute for Medical Research and Occupational Health (CROATIA) (prof. Aleksandra Fucic)
17. School of Medicine –CIPRO (Prof. Elpida-Niki Emmanouil-Nikoloussi)

PROGETTO NAZIONALE

18. Università degli Studi di Milano, Dipartimento di Salute Ambientale, Sezione Epigenetica - MILANO
19. Istituto Superiore di Sanità, Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare (Unità di Tossicologia Alimentare e Veterinaria) – ROMA
20. Istituto ZooProfilattico Sperimentale del Mezzogiono - PORTICI
21. Unità Operativa Complessa di Urologia - Ospedale San Camillo – Forlanini - ROMA
22. ENEA – Unità di Tossicologia – Radiologia, Biologia e Salute Umana – CASACCIA (Fr)
23. Università degli Studi di Brescia, Dipartimento di Epidemiologia e Urologia - BRESCIA
24. Università degli Studi di Modena e Reggio Emilia, Dipartimento di Urologia - MODENA
25. Università degli Studi di Pisa, Unità Operativa Complessa di Andrologia - PISA
26. ARPA Emilia Romagna, Dipartimento Ambiente-Salute – MODENA
27- Ospedale di GELA, Unità Operativa Complessa di Urologia – GELA
28. Centro HERA - CATANIA
27. Unità Territoriale di Urologia – PESCARA
28. Unità Operativa Complessa di Urologia- MARTINAFRANCA (TA)
29. Centro «CREA» TARANTO

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Azienda Sanitaria Locale Salerno

Bando Ministero della Salute

Direzione Generale della Prevenzione Sanitaria

Progetto FAST (Fertilità, Ambiente/Alimentazione, Stili di Vita)

Un modello di intervento per la prevenzione dell'infertilità in adolescenti sani residenti in aree a forte impatto ambientale

1) U.O.C. di Urologia, Ospedale "S. Francesco d'Assisi" Oliveto Citra, Ambulatorio Pubblico di Andrologia - ASL Salerno Coordinamento
2) Unità di Igiene, Epidemiologia e Sanità Pubblica e U.O. di Urologia, Università degli Studi di Brescia.
3) Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare, Istituto Superiore di Sanità, Roma

Unità di ricerca collaboranti

4) Istituto di Scienze dell'Alimentazione, Consiglio Nazionale delle Ricerche (ISA-CNR), Avellino
5) Laboratorio di Epigenetica, Università degli Studi di Milano
6) Laboratorio Microinquinanti, Istituto Zooprofilattico Sperimentale del Mezzogiorno Portici
7) Laboratorio di Biosicurezza e Stima del Rischio SSPT-TECS-BIORISC, ENEA CR Casaccia

Animazione, diffusione, comunicazione

Rete Nazionale EcoFoodFertility, Cittadinanza Attiva, Associazione Hera, ISDE Medici per l'Ambiente, Società Italiana di Andrologia, Società Italiana di Urologia, FIMMG, SIMPeSV

OBIETTIVO GENERALE

Applicare un modello operativo di prevenzione basato su modifiche dello Stile di Vita di tipo educativo-comportamentale e nutrizionale per la salvaguardia della qualità del seme e della fertilità maschile in 450 adolescenti sani 18-19enni, residenti in aree ad alto impatto ambientale e valutarne l'efficacia attraverso la misurazione di biomarcatori di dose efficace e di effetto nel sangue e nel seme.

Le aree individuate:**Aree ad alto impatto ambientale**, sulla base dei dati raccolti e divulgati dalle Agenzie Regionali di Protezione per l'Ambiente (ARPA):

Nord	Centro	Sud
Lombardia	**Lazio**	**Campania**
SIN Brescia-Caffaro	Valle del Sacco (FR)	Area Nord Provincia di Napoli

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ISTITUTO SUPERIORE DI SANITÀ

UNIVERSITAS STUDIORUM BRIXIAE

- **Questo studio si propone di applicare un intervento di prevenzione basato su modifiche dello Stile di Vita di tipo educativo-comportamentale e nutrizionale per la salvaguardia della qualità del seme e della fertilità maschile mediante un trial randomizzato controllato.**

- **Lo studio verrà effettuato su 450 adolescenti sani, di 18-19 anni di età, residenti in aree ad alto impatto ambientale, assegnati a caso a un gruppo sottoposto all'intervento e a un gruppo di controllo.**

- **L'efficacia dell'intervento sarà valutata attraverso il confronto tra i due gruppi, al termine del follow-up, dei livelli di biomarcatori di dose efficace (elementi in traccia) e di effetto (stato RedOx, antigene prostatico specifico PSA, esami epigenetici) nel sangue e nel seme.**

- **Quest'ultimo costituisce sia un sensibile biomarcatore sentinella della popolazione esposta che un indicatore di salute non solo riproduttiva.**

- **Tale modello permetterà di valutare l'efficacia dei cambiamenti dello Stile di Vita per contrastare gli effetti di inquinanti ambientali sulla salute riproduttiva maschile in diversi contesti ambientali e socio-culturali**

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UNIVERSITAS STUDIORUM BRIXIAE

Invertenti informativi nelle scuole e università aderenti e selezione degli studenti
Questionario iniziale per la selezione dei partecipanti allo studio di intervento (non fumatori, non uso di droghe, non bevitori abituali, non esposti professionalmente, patologie croniche e acute)

Studenti 18-20enni Area Brescia-Caffaro, Lombardia (n=150)

Studenti 18-20enni Area Valle del Sacco, Lazio (n=150)

Studenti 18-20enni Area Terra dei fuochi, Campania (n=150)

Valutazioni **al basale, tempo 0 (t = 0)**

visita uro-andrologica
analisi liquido seminale e sangue
valutazione dello stile di vita tramite una prima somministrazione dei questionari sull'alimentazione e sull'attività fisica.

Randomizzazione

Gruppo di intervento (n=75 per area = 225), percorso educativo nutrizionale e comportamentale sullo stile di vita

Gruppo di controllo (n=75 per area = 225), consegna di un opuscolo sullo stile di vita secondo linee guida

Valutazioni a 4 mesi (termine dell'intervento)

visita uro-andrologica
analisi liquido seminale e sangue
valutazione dello stile di vita tramite una seconda somministrazione dei questionari sull'alimentazione e sull'attività fisica.

Valutazioni a 8 mesi dal termine dell'intervento (follow-up)

- **visita uro-andrologica**
- **analisi liquido seminale**
- **valutazione dello stile di vita tramite una terza somministrazione dei questionari sull'alimentazione e sull'attività fisica.**

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Evaluation of environmental impact on sperm DNA integrity by Sperm Chromatin Dispersion Test and p53 ELISA. Preliminary data (ECOFOODFERTILITY Project)

Reproductive Toxicology 2015; 56: 20

Montano L, Notari T, Raimondo S, Bergamo P, Rossi M, Luongo D, Volpe MG, Iannuzzi L. Campania Region Group Research EcoFoodFertility

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Sperm Chromatin Dispersion Test

DNA Fragmentation Index (DFI)

+

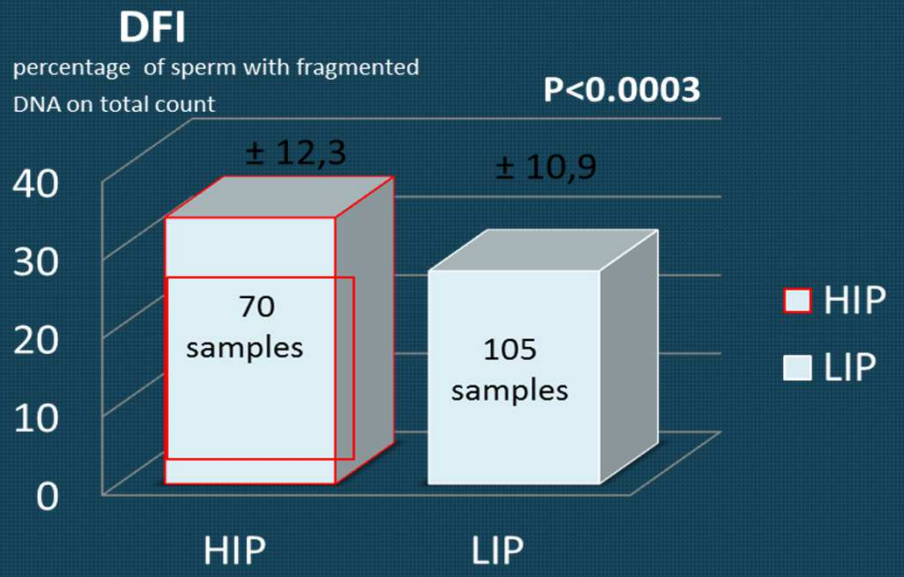
p53 Elisa

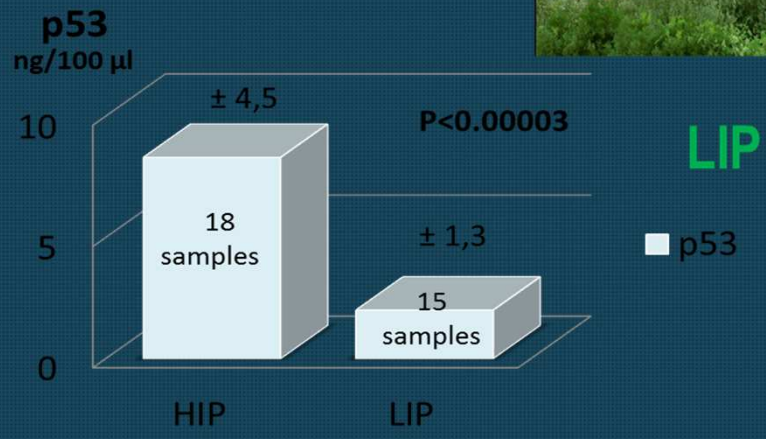
HIP

208 healthy males (mean age 30±4)
no-smoking, no-drinker, no professional exposed

LAND OF FIRES – HIP (88)

Alto-Medio Sele, Cilento – LIP (120)





Quantitative evaluation of p53 as a new indicator of DNA damage in human spermatozoa" Salvatore R. et al. J. Hum Repr Science 2014

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SOCIETÀ ITALIANA DI UROLOGIA

FONDATA NEL 1908

PREMIO «BRACCI» 2017
(migliore pubblicazione anno 2016) Congresso SIU Napoli 7-11 Ottobre 2017

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Human semen as an early, sensitive biomarker of highly polluted living environment in healthy men: A pilot biomonitoring study on trace elements in blood and semen and their relationship with sperm quality and RedOx status.

Reproductive Toxicology Sept. 66 (2016) 1–9

Paolo Bergamo, Maria Grazia Volpe, Stefano Lorenzetti, Alberto Mantovani,Tiziana Notari, Ennio Cocca, Stefano Cerullo, Michele Di Stasio, Pellegrino Cerino and **Luigi Montano***

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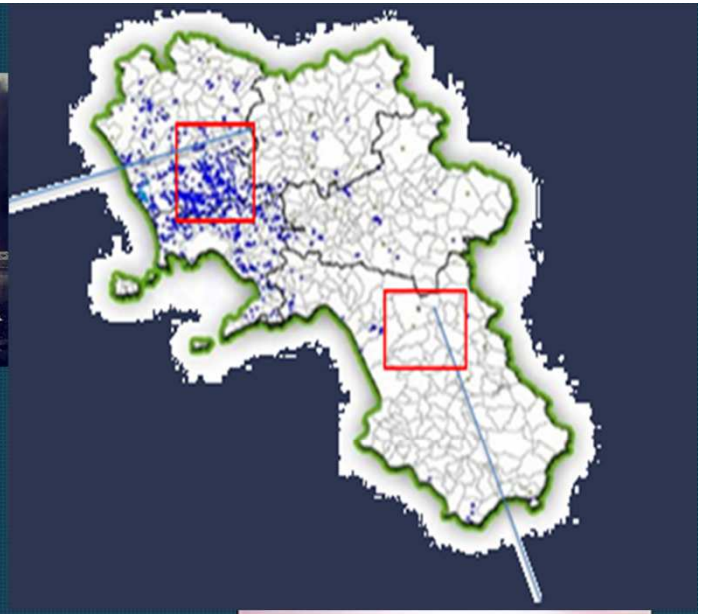


SUBJECTS From Campania Region (Southern ITALY)

110 **healthy men**, (**no smokers, no habitual drinkers, no professional exposed, no drug, no chronic deseases)**

TdF

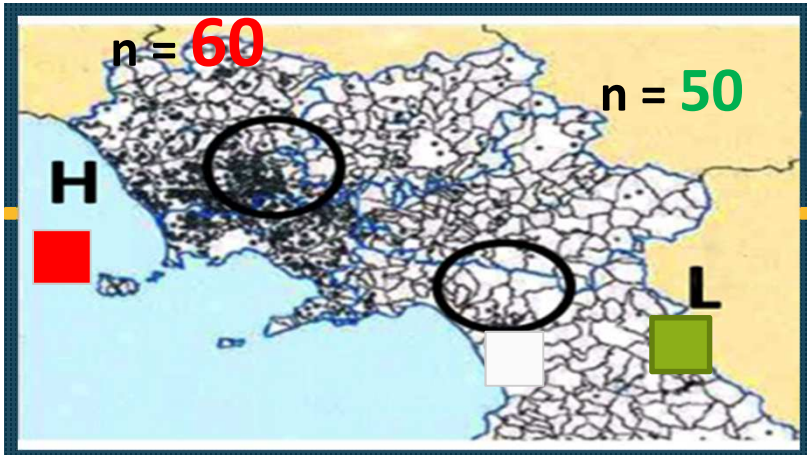
n = 60 living in «**Land of Fires**» (High Environmental Impact Area **TdF**),
age 28 ± 5,
BMI 24.5 ± 2.4

n = 50 living in Province of Salerno (Low Environmental Impact Area **SaS**)
age 28 ± 7
BMI 24.5 ± 2.4

SaS

ANALYSIS

1 - **Spermiogram**
2 – **Trace elements in semen and blood**
(**Al, As, Ba, Be Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, Zn)** by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES)
3 – **Total antioxidant capacity in semen and blood** (TAC)
In a randomly-selected subjects (n = 20) from each group
4 - **DNA Fragmentation Index (DFI) by Sperm Chromatin Dispersion (SCD-Halotech)**
5 - **Antioxidant enzymes in semen (GSR; GPx).**



BLOOD

Al	+ 63%
Mn	+59%
Cr	+51%
Mg	+41%
Li	+36%
Co	+33%
Ca	+26%

Blood RedOx Status
(Vit E, Vit A, Tot Antiox Capacity)

=

SEMINAL PLASMA

Cr + 95 %
Fe - 87 %
Cu + 45 %
Zn + 8 %

Transition metals

Semen RedOx Status
(GSH, Tot. Antiox. Capacity)

- 30%

Antioxidant enzymes
(GSR, GSPx)

- 30%

Sperm Motility

- 20%

DNA Sperm damage
(SCD)

+ 45%

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	SaS	TdF	p value
	(n= 50)	(n= 60)	
Semen quality parameters			
pH	7.9 ± 0.1	7.9 ± 0.2	N.S.
Volume (mL)	2.9 ± 0.9	3.0 ± 1.3	N.S.
Cell concentration (10^6/mL)	42.8 ± 18	44.2 ± 26	N.S.
Total sperm number (10^6) (10^6/ejaculate)	132.1 ± 87	140 ± 89	N.S.
Progressive motility (%)	35.0 ± 13.4	32.3 ± 12.7	N.S.
Morphology (%)	6.4 ± 2.4	6.0 ± 2.3	N.S.
Total motility (%)	55.2 ± 19.3	48.5 ± 16.8	0.028
Round cells conc. (10^6/mL)	2.5 ± 2.1	2.6 ± 1.8	N.S.
Blood RedOx status			
TAC (mM)	3.5 ± 1.0	3.1 ± 1.2	N.S.
SH (mmoles/ mg prot)	58.7 ± 7	62.3 ± 11	N.S.
Vitamin A (µmol/L) (mmoles/L)	2.6 ± 0.7	2.4 ± 0.7	N.S.
Vitamin E (µmol/L) µmol/L (mmoles/L)	32.1 ± 1.0	30.7 ± 8.8	N.S.
Seminal plasma RedOx status			
TAC (mM)	1.2 ± 0.4	0.9 ± 0.3	<0.005
SH (mmoles/mg)	212.5 ± 66	207.3 ± 60	N.S.
GSH (nmoles/mg/min)	0.5 ± 0.1	0.3 ± 0.0	<0.01
GSSG (nmoles/mg/min)	0.2 ± 0.3	0.1 ± 0.5	N.S.

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		TdF	SaS
GSR p <0.05	(nmoli NADPH/mg/min)	26.2 ± 8.4	37.0 ± 5.2 *
GPx P <0.05		202.5 ± 55	269.4 ± 55 *
GSH p <0.01		0.5 ± 0.1	0.3 ± 0.0
DFI p <0.05	(%)	27.7 ± 13.3	13.8 ± 7.1 *

		TdF	SaS
Total Motility spermatozoa p < 0.05	%	48.5 ± 17	55.2 ± 19 *
TAC (Blood)	(mM)	3.1 ± 1.0	3.5 ± 1.2
TAC (seminal plasma) p < 0.05	(mM)	0.9 ± 0.4	1.2 ± 0.3 *

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HIGHLIGHTS:

- Trace elements in semen and blood discriminate people living in polluted areas
- Seminal plasma is more sensitive than blood to redox status alteration
- Lower sperm quality and antioxidant defences associated to highly polluted area
- In healthy males, semen is proposed as early biomarkers of environmental pollution

CONCLUSION

- The findings on spermatozoa suggest a direct effect of environment on spermatogenesis and/or sperm integrity;
- The lower semen RedOx status (GSH and TAC) and activity of antioxidant enzymes suggest a reduced ability for antioxidant response in sperm from the HIGH impact area.
- The combined measurement of trace elements in blood and semen in association with the overall assessment of semen quality, RedOx parameters and sperm DNA damage, allow to investigate the potential link between environmental pollutants and semen quality in healthy males.
- Taken together, the sperm and RedOx findings indicate a possible pattern of "early semen biomarker" for healthy adult males living in the "Land of Fires", eventually transferable to other pollution scenarios

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Molecular Sciences

Effects of Highly Polluted Environment on Sperm Telomere Length: A Pilot Study.

Int J Mol Sci. 2017 Aug 4;18(8)

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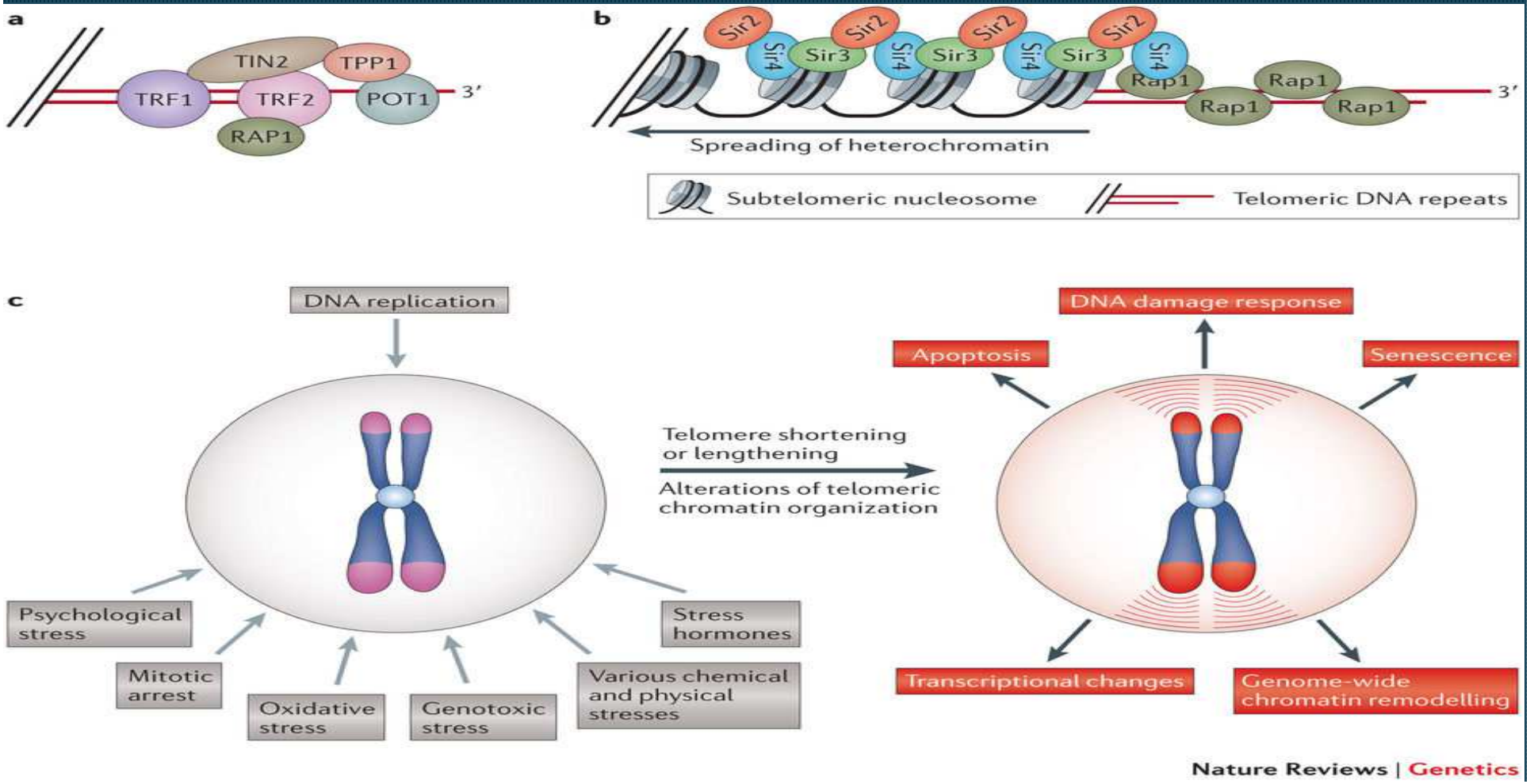
Telomere is a region of repetitive DNA at the end of a eukaryote chromosome, maintain chromosome stability and genome integrity and have multiple functions, including regulating the reproductive lifespan. Furthermore environment, lifestyle factors and psychological stress play crucial role in modulating telomere length.



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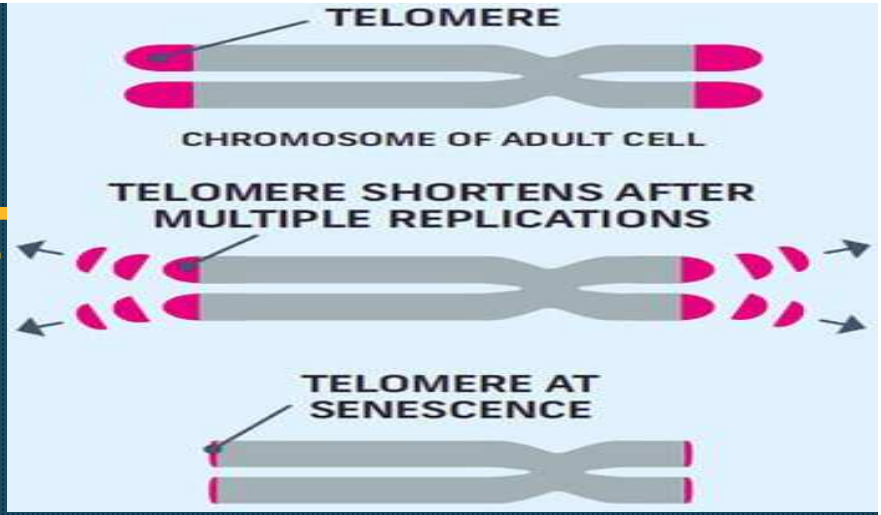
Telomerase activity is low for somatic and high for germ, staminal and cancer cells, in fact telomere shortens in somatic cells are (e.g. leucocytes) is associated with senescence



Anyway both shortening and lengthening of telomere length (TL) have been observed in a number of clinical conditions including cardiovascular disease and cancer
[Calado, R.T.; Young, N.S. Telomere diseases. N. Engl. J. Med. 2009, 361, 2353–2365]

Interestingly, accumulating evidence indicates that leukocyte telomeric DNA may be one important target of environmental pollutants [Hoxha M et al. 2009, Shin JY et al. 2010, Hou L et al. 2012, Gao J et al. 2015 , Ling X et al. 2016,]

In fact, an increase in telomere length linked to exposure has been evidenced in blood.
Specifically, Shin et al. [Mutagenesis 2011] found that low-dose persistent organic pollutants (POPs) increased LTL.

Similarly, Dioni et al. [Environ. Health Perspect. 2011] ,reported longer LTL associated with short-term exposure to particulate matter in a group of steel workers, likely due to an acute response of inflammatory cells and a positive association between the level of arsenic and telomere length has been also established [Li H et al. 2012 - Gao J et al. 2015]

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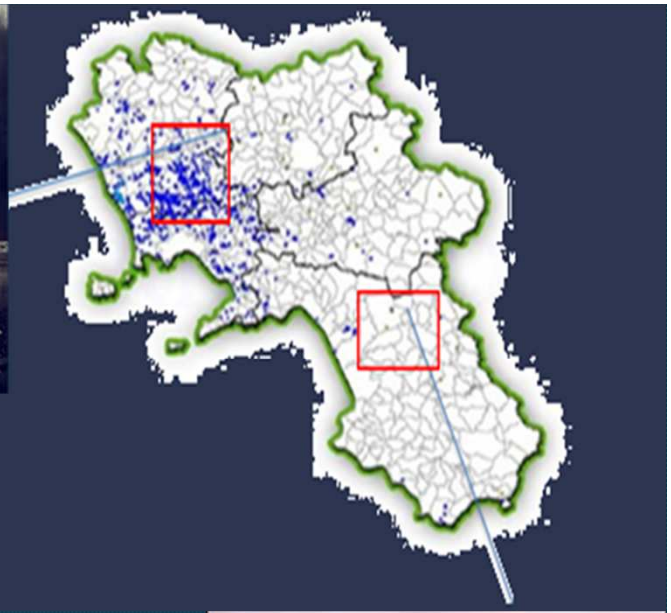

TdF

In order to evaluate the influence of environmental exposure on TL in both leukocytes (LTL) and sperm cells (STL) we recruited 112 clinically healthy, normospermic men living in two areas of Campania region (Southern Italy) with high (n = 57, High Group TdF) or low (n = 55, Low Group) environmental pressure

	Low group (n=55)	High group (n=57)	p-value
Age (years ± SD)	29.8 ± 5.4	28.1 ± 5.8	ns
BMI	25.2 ± 3.4	25.1 ± 3.6	ns
Smoking status, n	17	14	ns
Semen quality parameters			
Volume (mL)	3.1 ± 1.1	3.1 ± 1.4	ns
Cell concentration (10^6/mL)	54.7 ± 23.6	54.5 ± 26.2	ns
Total sperm number (10^6/ejaculate)	172.7 ± 101.9	154.2 ± 97.9	ns
Morphology (%)	6.3 ± 1.9	6.3 ± 2.0	ns
Sperm Motility			
Progressive Motility (%)	29.6 ± 14.3	33.0 ± 12.4	ns
Non-Progressive Motility (%)	18.6 ± 7.5	25.8 ± 17.2	0.005

SaS

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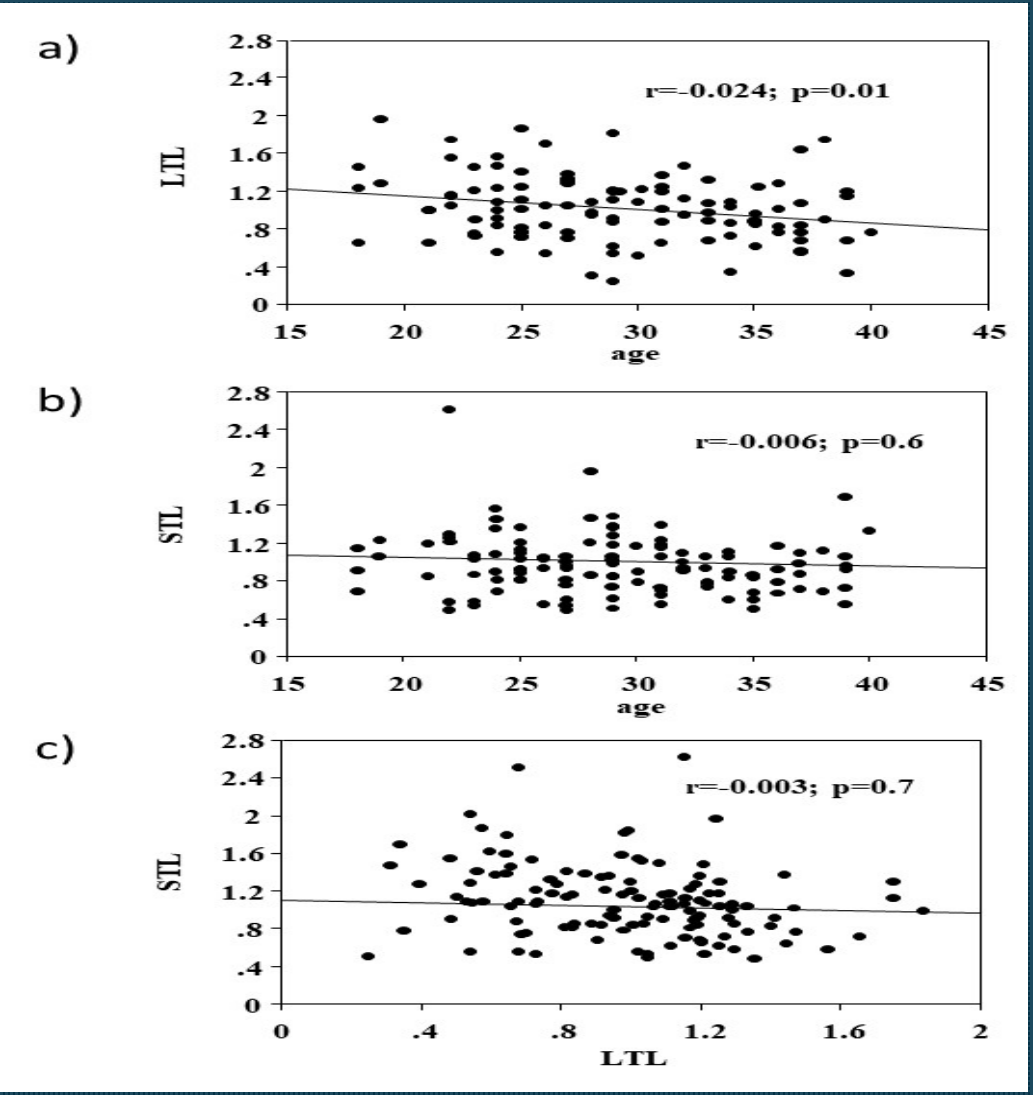
a)
leucocyte telomere length (LTL) and age,

b)
sperm telomere length (STL) and age

C)
LTL and STL



Relationship

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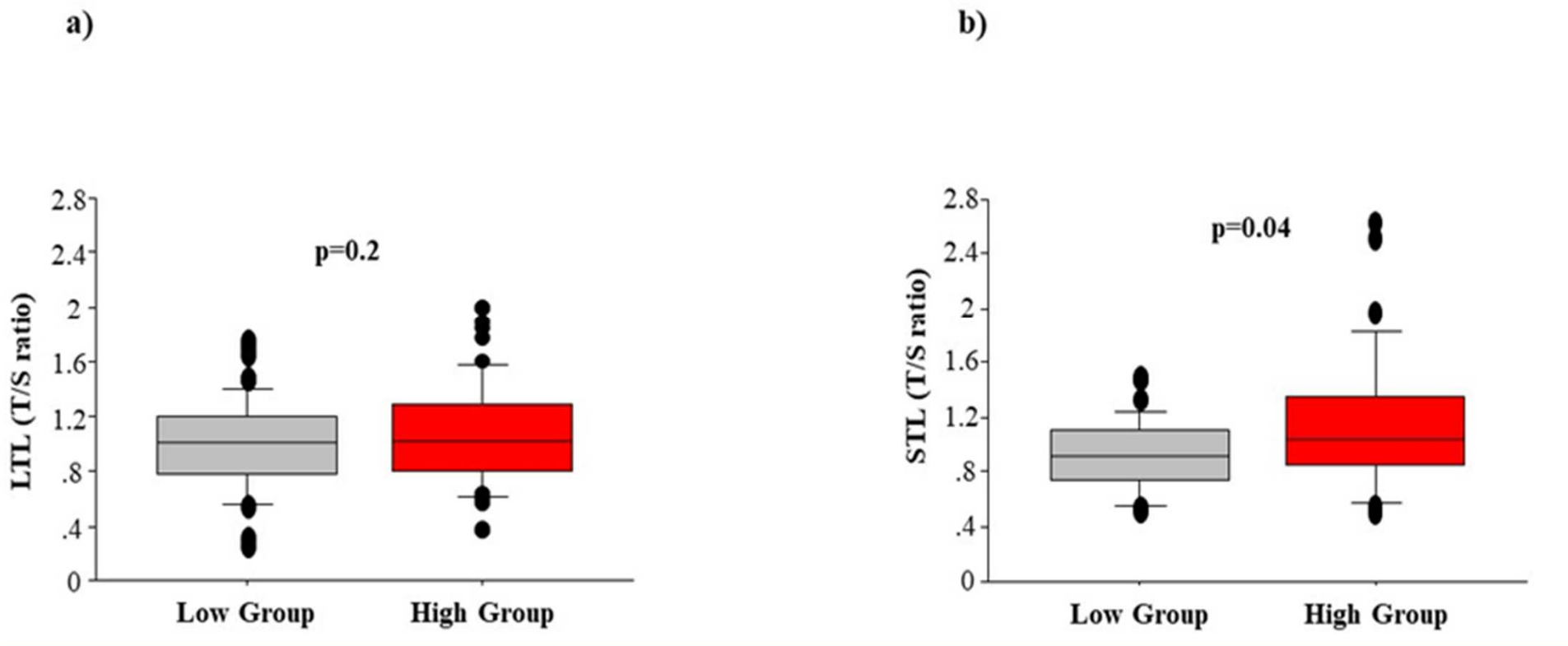

Association between high or low impact of exposure (High Group vs Low Group) and leucocyte telomere length (LTL) and b) semen telomere length (STL)

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1) Considering that a relationship between dysfunctional telomere length and increased cancer risk has been observed [IHou, L et al. Cancer Lett. 2012] in particular, longer telomeres have been associated with some types of cancers, including melanoma and lung cancer [Rode, L Int. J. Epidemiol. 2016];
2) A very recent Mendelian randomization study of 83 non-communicable diseases, including 420,081 cases and 1,093,105 controls, showed that longer telomeres were associated with increased risk of several cancers, but also with reduced risk of some non-neoplastic diseases, including cardiovascular diseases [Haycock PC et al. JAMA Oncol. 2017]
3) We can assume that in an area where is recognized a higher incidence of cancer especially lung cancer and other in young population, the telomerase activity might be altered and in some subjects more susceptible the findings of this slight difference in sperm telomere length might significate an higher risk of cancer
4) How calculate this risk is obviously difficult,but these findings should be promote an higher health surveillance in this area

CONCLUSION

These findings support the view that semen is a sensitive sentinel biomarker of environmental exposure. Further studies in larger populations are needed to understand the significance of telomere lengthening in areas of high environmental crisis.

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First point

Semen RedOx status, motility, SDF and STL

can be considered as early markers of environmental pollution and human semen seems an earlier and sensitive source of biomarkers than blood to monitor high environmental pressure on human health, hence useful for innovative prevention programs and health surveillance in risk areas

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The role of the Mediterranean diet on sperm morphology in healthy men living in polluted area (EcoFoodFertility Project).

Montano Luigi[1], Porciello Giuseppe[2], Anna Crispo[2], Lorenzetti Stefano[3], Raimondo Salvatore[4], Ubaldi Stefania[5], Caputo, Michela[6]

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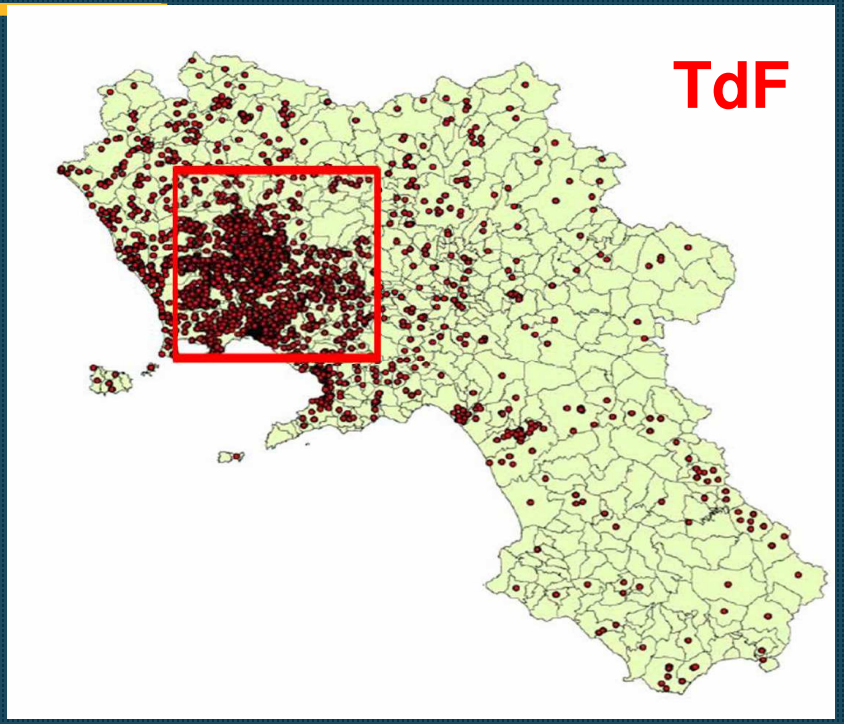
CAMPIONE ANALIZZATO

94 maschi sani

Non fumatori, non bevitori abituali, non professionalmente esposti
Età media 28 ± 6,

n = 94 residenti in comuni tra Napoli e Caserta (ALTO impatto ambientale; **Terra dei Fuochi**)



VALUTAZIONE DELLO STATO NUTRIZIONALE e ADERENZA ALLA DIETA MEDITERRANEA

- Food Frequency Questionnaire
- **14-item PREDIMED questionnaire**

SPERMIOGRAMMA

Appendix Table 1. **Quantitative Score of Adherence to the Mediterranean Diet**

Foods and Frequency of Consumption	Criteria for 1 Point*
1. Do you use olive oil as main culinary fat?	Yes
2. How much olive oil do you consume in a given day (including oil used for frying, salads, out-of-house meals, etc.)?	≥4 tbsp
3. How many vegetable servings do you consume per day? (1 serving = 200 g [consider side dishes as half a serving])	≥2 (≥1 portion raw or as salad)
4. How many fruit units (including natural fruit juices) do you consume per day?	≥3
5. How many servings of red meat, hamburger, or meat products (ham, sausage, etc.) do you consume per day? (1 serving = 100–150 g)	<1
6. How many servings of butter, margarine, or cream do you consume per day? (1 serving = 12 g)	<1
7. How many sweet or carbonated beverages do you drink per day?	<1
8. How much wine do you drink per week?	≥3 glasses
9. How many servings of legumes do you consume per week? (1 serving = 150 g)	≥3
10. How many servings of fish or shellfish do you consume per week? (1 serving = 100–150 g of fish or 4–5 units or 200 g of shellfish)	≥3
11. How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, biscuits, or custard?	<3
12. How many servings of nuts (including peanuts) do you consume per week? (1 serving = 30 g)	≥1
13. Do you preferentially consume chicken, turkey, or rabbit meat instead of veal, pork, hamburger, or sausage?	Yes
14. How many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with *sofrito* (sauce made with tomato and onion, leek, or garlic and simmered with olive oil)?	≥2

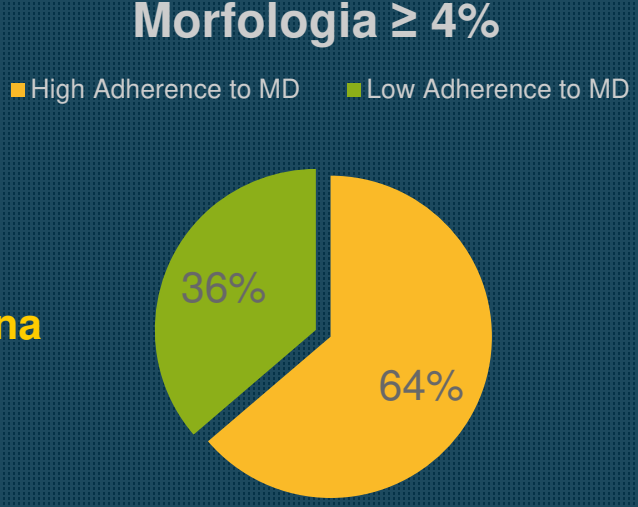
* 0 points if these criteria are not met.

PREDIMED SCORE

< 7 Low Adherence to the MD

≥ 7 High Adherence to the MD

In totale 54 uomini presentavano una buona aderenza alla DM, rispetto a 40 che avevano una aderenza più scarsa



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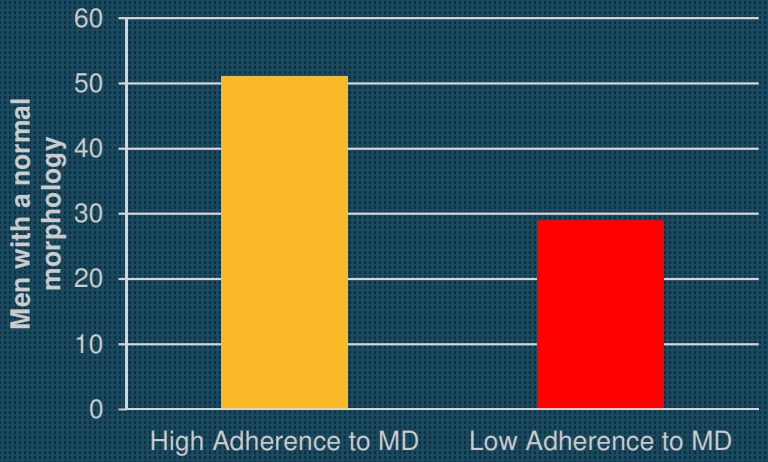
Una maggiore aderenza alla DM si associava a una morfologia nemaspermica migliore



	Low Adherence	High Adherence
Morph <4%	11 (27,5%)	3 (5,6%)
Morph ≥ 4%	29 (72,5%)	51 (94,4%)

È stata riscontrata una differenza significativa tra uomini con alta aderenza e uomini con bassa aderenza alla DM sulla morfologia spermatica (94,4% vs 72,5%, p=0,003)

Elevato consumo di vegetali caratteristico di un regime dietetico di tipo Mediterraneo ricco in antiossidanti potrebbe sia controbilanciare l'eccessiva produzione di ROS indotta da inquinanti, sia promuovere la detossificazione enzimatica e proteggere la qualità del seme dagli inquinanti.

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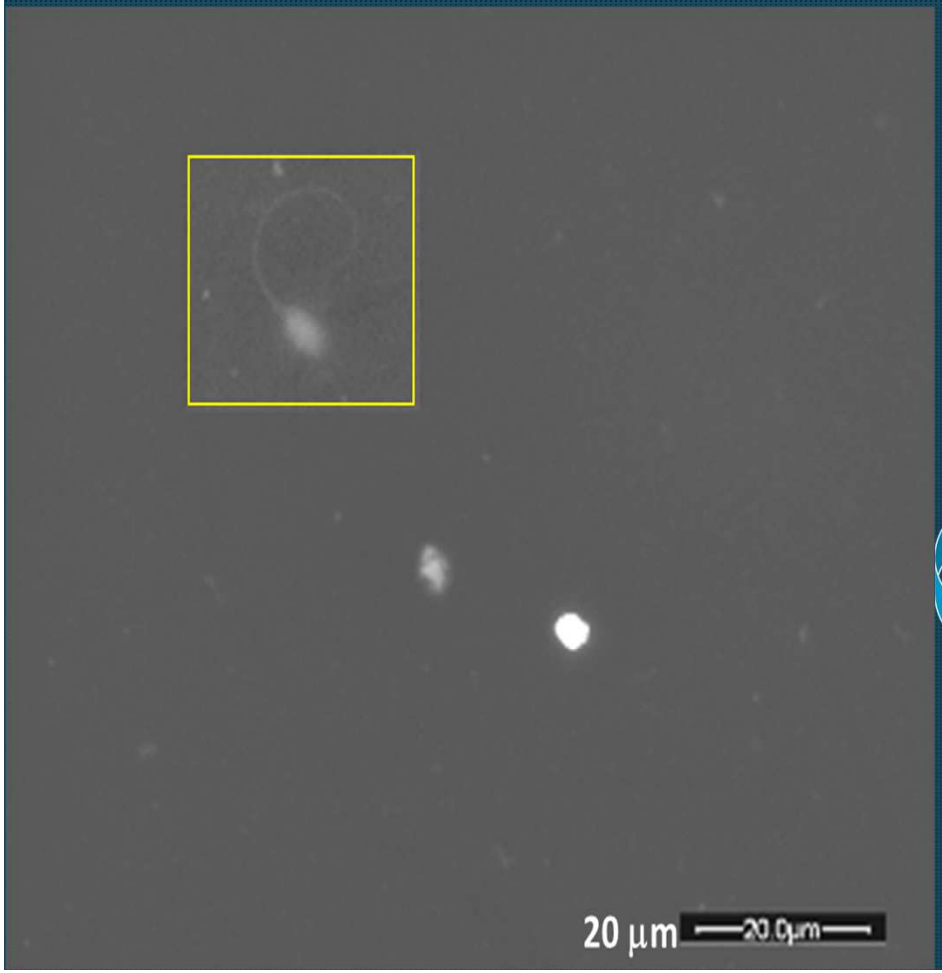
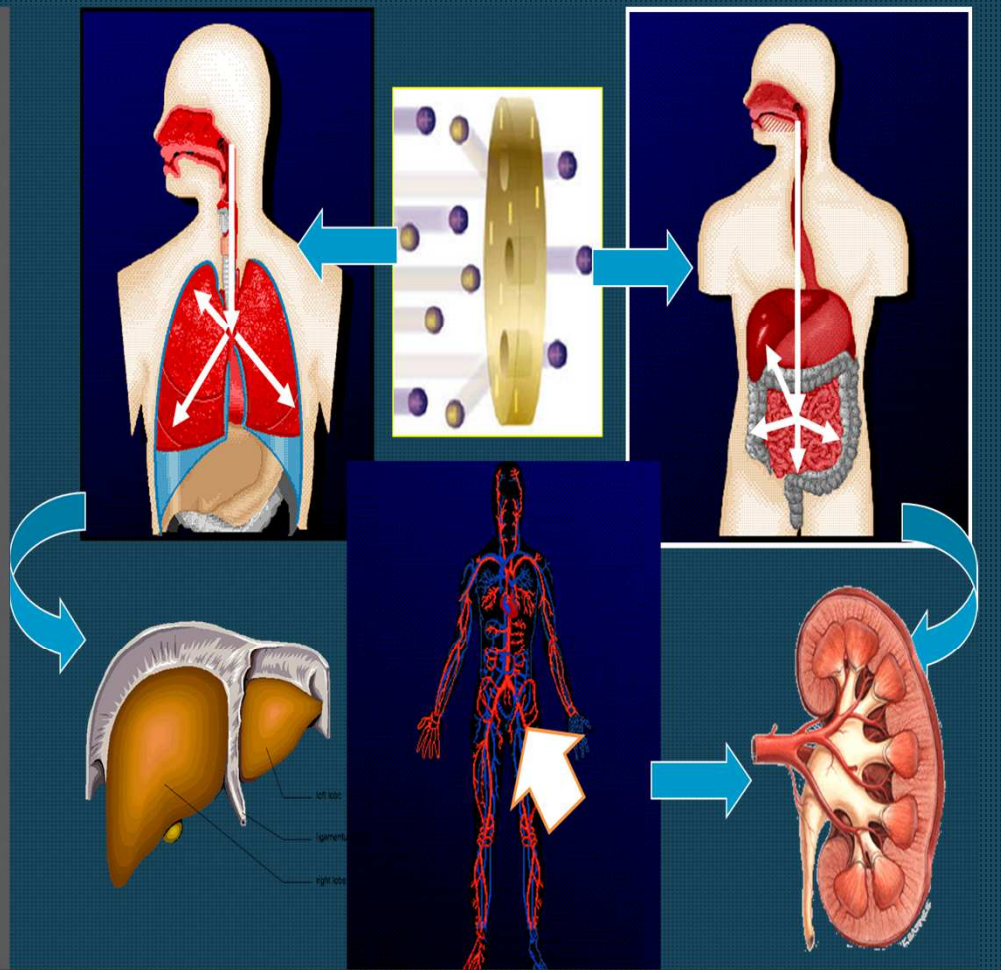
Antonietta Gatti
Luigi Montano

Innovative environmental scanning electron microscopy observations of the human semen in the study of male infertility (preliminary data)

Reproductive Toxicology, Volume 72, September 2017, Page 38





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NANODIAGNOSTICS

European Teratology Society

45th Annual Meeting 2017 - Budapest, Hungary • 4th to 7th September 2017

Innovative Environmental Scanning Electron Microscopy observations of the human semen in the study of male infertility (Preliminary data)

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INTRODUCTION

Male infertility is increasing dramatically in the industrial world, becoming a public health top priority. It affects an estimated 15% of couples globally, amounting to 48.5 million couples and one third of cases can be due to a male factor. Semen analysis is a common, suitable measure of assessing the male fertility. We investigated human semen from a new point of view: the involvement of environmental (polluted area, war-field) factors and the suspected increased incidence of male-related infertility.

RESULTS

In the cases of soldiers exposed to the bombs-target explosions we found metallic particles (Iron, stainless steel, tungsten, bismuth, titanium) in quantity statistically meaningful related to the other groups. Strange Antimony-Cobalt particles were found in a case of a soldier involved in training activity in a firing range where new bombs were tested. In the cases of subjects living in "Land of Fires", we found particles of Silicates, Calcium-Alluminum-Silicon, Calcium-phosphate in seminal plasma and several adherent to the sperm cells.
All samples presented low sperm motility. Also patients affected by cancer, the semen appeared polluted but less than other two groups
These preliminary data, demonstrated that environmental pollution if inhaled can be dispersed in the human body, and particulate matter can reach testicles and contaminate the semen, directly correlating with the subject's exposure. The particles arenot biocompatible, some can be toxic and induce the spermatozoa death, but their presence can affect sperm function as the sperm motility.

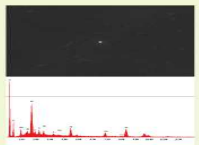


Fig. 1
Tungsten-titanium nanoparticle

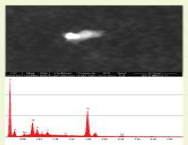


Fig. 2
Particle of Titanium-Aluminum

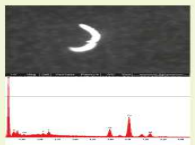


Fig. 3
Particle of Stainless steel

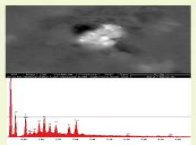


Fig. 4
Particle of Sodium-Phosphorus-Silicon--Calcium-Chlorine-etc. It is a combustive origin particle

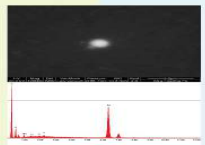
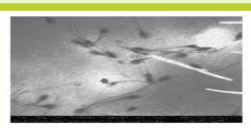
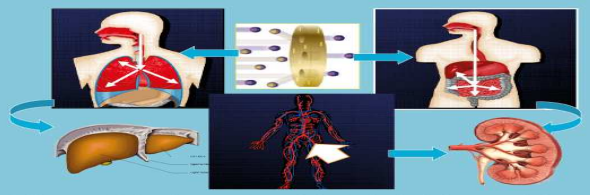


Fig. 5
Particle of combustive origin of Iron



MATERIALS AND METHODS

The study investigated directly human semen of exposed people by means of the Environmental Scanning Electron Microscopy (ESEM) and the x-ray microprobe of an Energy Spectroscopy [1]. The study involved 19 cases of soldiers exposed to a war-field pollution, to 8 cases of male healthy, no smoking, no habitual alcohol drinking, no occupational exposure, living in "Land of Fires", an area ill-famed for the multiplicity of sources of pollution in Southern Italy recruited within EcoFoodFertility project (www.ecofoodfertility.it) [2-3] and in other 7 cases of patients affected by tumours (non Hodgkin's Lymphoma). Three different techniques were used (High vacuum, low vacuum and room pressure) to study the human semen in order to identify micro, submicro and nanosized pollution and to identify the chemical composition of the particulate matter identified.
The seminal fluids were donated to Nanodiagnostics Laboratory to be observed under Field Emission Gun Environmental Scanning Electron Microscope (ESEM Quanta 250, FEI Company, The Netherlands) coupled with an X-ray microprobe of an Energy Dispersive Spectroscope (EDS by Edax, USA) to get the elemental analysis of the particles identified in the tissue.
All elements beyond Beryllium can be detected.
ESEM can work at room conditions and can be used to examine uncoated, non-conductive and wet specimens, and do not need any dehydratation of the biological sample. Different kinds of observation were carried out: high-vacuum (10-6 Torr), low-vacuum (0.2-1.2 Torr) and air; secondary and backscattered electron mode; accelerating voltages from 12kV to 25kV; point-to-point resolution from 6.0 to 4.0 nm. This new specific equipment was deemed to be necessary since, enabling to observe unprocessed samples, it ensures that no foreign particulates or air-borne dust are introduced after performing the biopsy. Usually the samples were embedded in paraffin, so 10-micron -thick sections were cut by means of a microtome (Leitz, Germany) and deposited on a polyester substrate. Then the section was deposited on an Aluminium stub with an adhesive carbon disc and a cellulose filter and put in the ESEM.



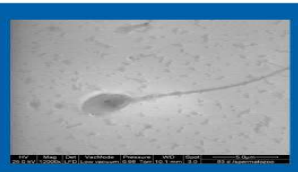
Scheme of the dispersion of the inhaled dust and dispersion inside the body included the testicles

Tab.1 List of the cases examined

- *19 Soldiers exposed to a warfield pollution*
- *8 cases of patients living in "Land of Fires" Southern Italy*

CONCLUSIONS

These preliminary data, demonstrated that environmental pollution if inhaled can be dispersed in the human body, and particulate matter can reach testicles and contaminate the semen, directly correlating with the subject's exposure. The particles can be not biocompatible, some can be toxic and induce the spermatozoa death, but their presence can affect sperm function as the sperm motility.



Acknowledgments:
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OBIETTIVI RAGGIUNTI: Al momento è stata completata la fase di reclutamento e di prelievo ematico a **4200 SOGGETTI (MASCHI E FEMMINE)**

FASE SUCCESSIVA: SEME UMANO: 500 maschi sani (20-40 anni) nelle aree di campionamento secondo il protocollo «**ECOFOODFERTILITY**» con relativo protocollo nutrizionale (**Bonifica Naturale dell'Uomo**)

ALTO IMPATTO

MEDIO IMPATTO

BASSO IMPATTO

Natural breaks

SPES
STUDIO DI ESPOSIZIONE NELLA POPOLAZIONE SUSCETTIBILE

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CONCLUSION

1) With a strong focus on the future of communities, male Reproductive System represents a "Sentinel Organ".

2) Biomarkers of reproductive health should be exploited as early signals of environmental pressure and increased risk of adverse chronic health effects so that the use of "***human seminal model***" might be the main objective to be considered in the agenda of public prevention policies for early detection and innovative programs of health surveillance in environmental risk areas.

All operators of human reproduction must consider a new role of Fertility, such as basis of Primary Prevention, not only for reproductive diseases, but also for chronic diseases of this and future generations, projecting Fertility into a wider dimension for the protection of public health!

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This research, supported by several environmental groups of the territory and involving different public research institutions, represents an example of active citizenship and scientific innovation. It was born as a response to an environmental and health crisis in the "Land of Fires", an area of Southern Italy ill-famed for the multiple sources of pollution. "EcoFoodFertility" responds to the need of clarity in the face of contradicting findings and media reports which generated a great deal of distrust in the healthcare system as well as anxiety within the population. It's a biomonitoring study, expanding to other risk areas of Italy and Europe. It studies the alterations of human semen, "environmental and health marker", as a key to understand both the level of environmental quality and its long term modifications, to set out early health risks for populations in relation with their living environment, diet and lifestyle. Currently the the project is in progress but this "research model" has already contributed to the development of other studies and initiatives by regional institutions and also to build a nationwide network of action in other environmentally-challenged areas.

Luigi Montano, MD, UroAndrologist, President of Italian Society of Human Reproduction has his expertise in Environmental Pathology and LifeStyle Medicine and his interest is focused on the reproductive and environmental health. In fact he is the creator and Coordinator of EcoFoodFertility project is starting up in several environmental risk areas.

Luigi Montano

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