SPA
SOCIEDADE PORTUGUESA
DE ANDROLOGIA, MEDICINA SEXUAL E REPRODUÇÃO

7OS ENCONTROS DE ANDROLOGIA

REABILITAÇÃO EM ANDROLOGIA E MEDICINA SEXUAL

AUDITÓRIO DO HOSPITAL DE STO ANDRÉ - LEIRIA

16.11.2019

LEUCOSPERMIA RECORRENTE: Tratar Até Quando?

Centro Hospitalar Cova da Beira, EPE

SPA
SOCIEDADE PORTUGUESA
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Bruno Jorge Pereira, MD, FEBU, FECSM

INFERTILIDADE

- Factor Masculino: 20-50%
- Infecções Genitourinárias: até 15% Infertilidade Masculina
- **Leucocitospermia:** indicador de MAGI's, inflamação ou auto-imunidade
- **OMS, 2010: >1 milhão leucócitos/mL**

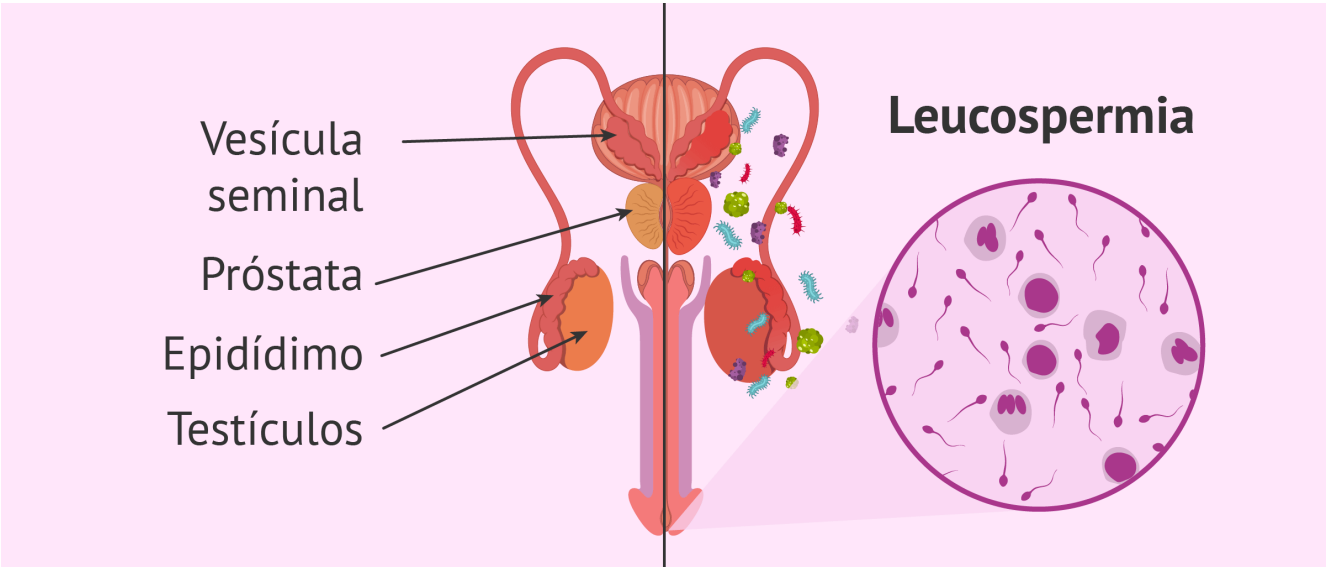


Table V Summary of generally accepted causal factors and additional potential contributing factors on severe male factor infertility ordered according to the severity of the effect on sperm parameters and fertility potential.

Classification	Subclass	Factor[a]	Fertile men (%)	Infertile men (%)	Effect among infertility patients
Causal factors	Absolute	Secondary hypogonadism	0	1.3	100% aspermia, azoospermia or cryptozoospermia
		Seminal tract obstruction	0	5.9	99% aspermia, azoospermia or cryptozoospermia
		Known genetic causes[b]	0	7.8	87.4% aspermia, azoospermia or cryptozoospermia
	Severe	Oncological diseases	0.3	1.6	62.7% aspermia, azoospermia or cryptozoospermia 37.3% oligozoospermia
		Severe sexual dysfunction	0	4.4	55.3% aspermia, azoospermia or cryptozoospermia 44.7% oligozoospermia
	Plausible	Congenital anomalies in uro-genital tract	1.8	10.7	40.3% aspermia, azoospermia or cryptozoospermia 59.7% oligozoospermia
		Acquired or secondary testicular damage	1.2	6.6	21.9% aspermia, azoospermia or cryptozoospermia 78.1% oligozoospermia
Potential contributing factors	Testicular health	Varicocele, Grade 2-3	12.0	26	Increased prevalence in idiopathic infertility and 'Other testicular factors' groups. Increased prevalence among all patients with detectable ($n > 0$) sperm counts, i.e. except for aspermia and azoospermia.
		Leukocytospermia	7.4	13.5	Increased prevalence in idiopathic infertility and 'Seminal tract obstruction' groups. Increased prevalence only in oligozoospermia cases.
	General health	Chronic disease	9.8	24.4	Increased prevalence in idiopathic infertility, 'Genetic causes', 'Congential anomalies in uro-genital tract' and 'Other testicular factors' groups. Contribution to aspermia excluded.
		Overweight (Obesity)	48.6 (13.5)	60.4 (22.0)	Increased prevalence in idiopathic infertily and in the majority of the causal factor subgroups.

[a]Included clinical diagnoses are detailed in Table II.
[b]Autosomal and sex chromosomal abnormalities, Y-chromosomal micordeletions, CTFR gene mutations.

Human Reproduction, Vol.32, No.1 pp. 18–31, 2017
Advanced Access publication on November 17, 2016 doi:10.1093/humrep/dew284

human reproduction ORIGINAL ARTICLE *Andrology*

Causes of male infertility: a 9-year prospective monocentre study on 1737 patients with reduced total sperm counts

M. Punab[1,*], O. Poolamets[1], P. Paju[1], V. Vihljajev[1], K. Pomm[1], R. Ladva[1], P. Korrovits[1], and M. Laan[2]

[1]Andrology Center, Tartu University Hospital, 50406 Tartu, Estonia [2]Human Molecular Genetics Research Group, Institute of Molecular and Cell Biology; Institute of Biomedicine and Translational Medicine, University of Tartu, 51010 Tartu, Estonia

LEUCOCITOSPERMIA

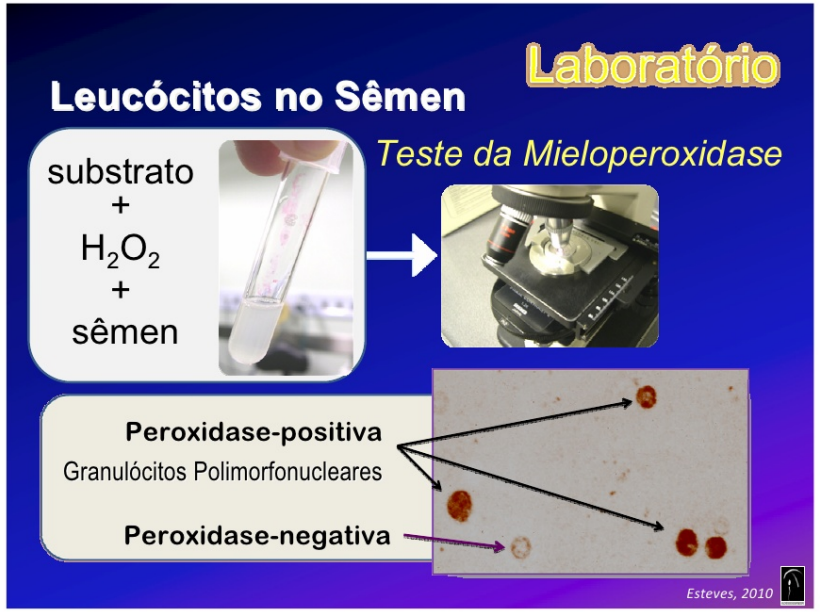
- Deformidade espermática elevada
- Dano acrossómico
- Defeitos da peça central
- Deformidades da cauda

LEUCOCITOSPERMIA?

- Que metodologia utilizar na contagem dos leucócitos seminais?
  1. OMS recomenda teste da peroxidase... Sensibilidade 47-60%
  2. Métodos imunocitoquímicos
  3. Citometria de fluxo

- Que limiar considerar para se definir leucocitospermia?
  - OMS, 2010: >1 milhão leucócitos/mL

- 50% dos casos com resolução espontânea?
- Vale a pena tratar?
- Ausência de tratamento específico...



LEUCOCITOSPERMIA?

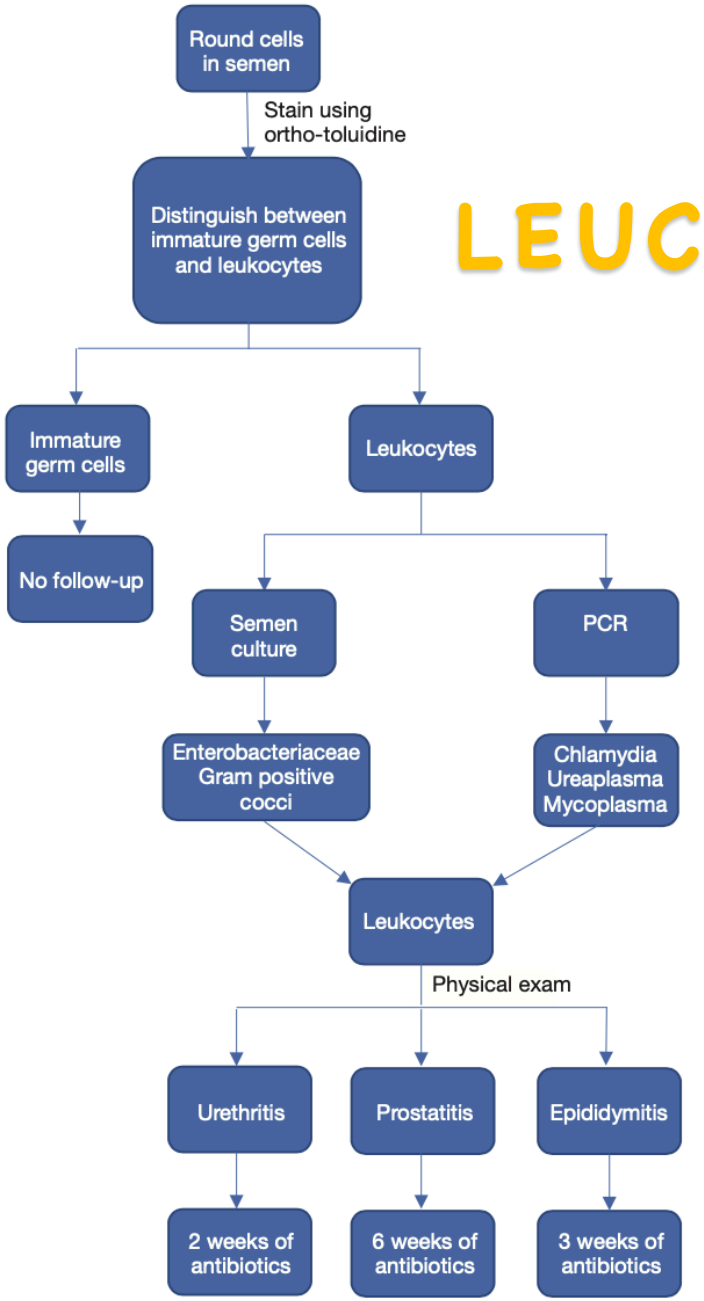


Figure 1 Male infertility work-up when infectious physical exam findings present.

ANDROLOGY

Differential expression of novel biomarkers (TLR-2, TLR-4, COX-2, and Nrf-2) of inflammation and oxidative stress in semen of leukocytospermia patients

Andrology, 2015, 3, 848–855

[1]S. Hagan, [1]N. Khurana, [1]S. Chandra, [1]A. B. Abdel-Mageed, [2]D. Mondal, [1]W. J. G. Hellstrom and [1]S. C. Sikka

[1]Department of Urology, and [2]Department of Pharmacology, Tulane University School of Medicine, New Orleans, LA, USA

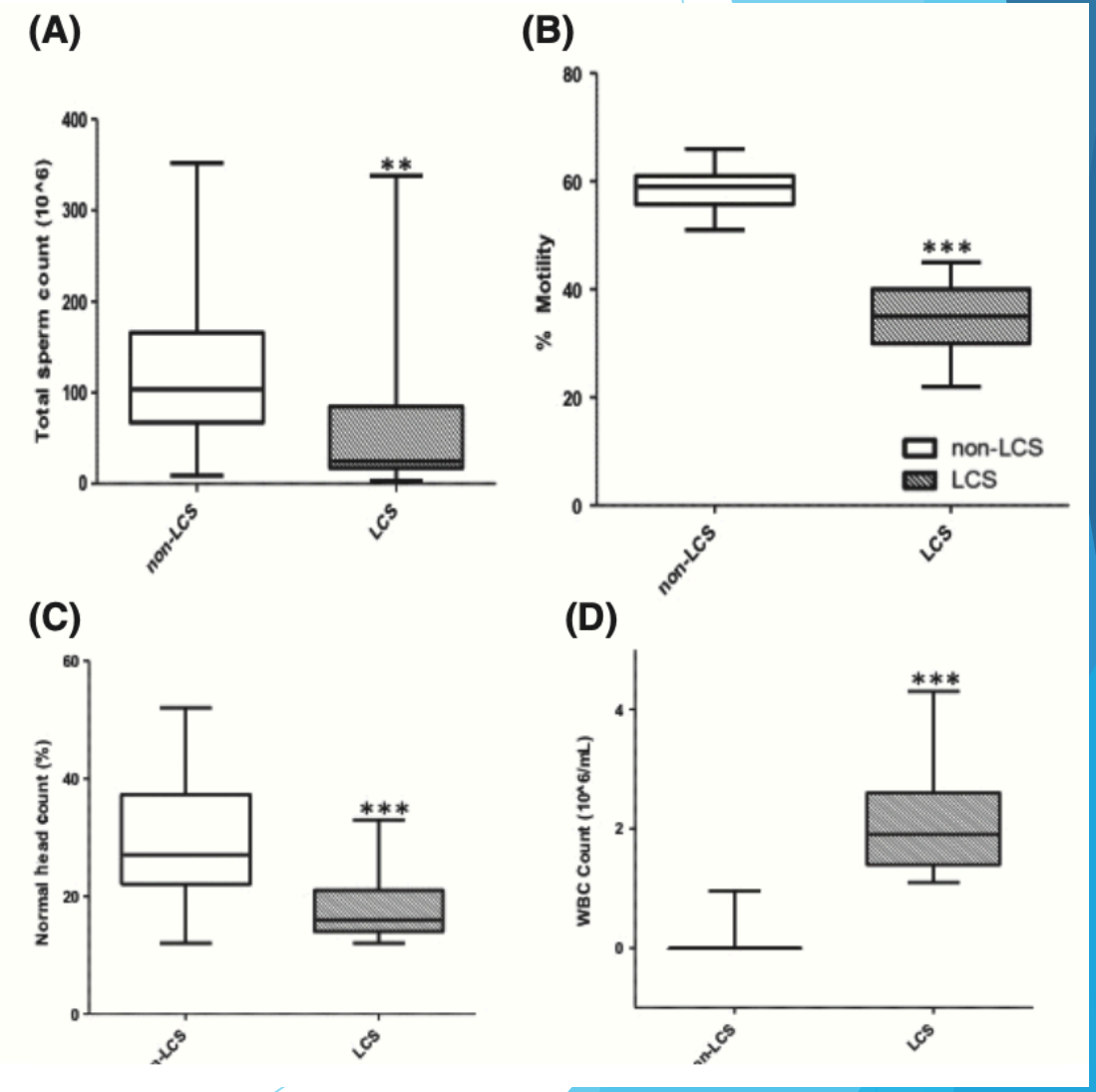


Figure 1 Measurement of semen parameters. (A) Total sperm count (10^6), (B) % motility, (C) % normal heads, and (D) WBC count (10^6/mL) were determined in semen samples taken from both non-LCS (n = 38) and LCS (n = 47) samples. Box plot indicates effect of LCS on semen parameter. Plots were scaled by dividing each median value. Statistical analysis was performed by the Mann–Whitney test. $p < 0.05$ was considered statistically significant (***$p < 0.0001$, **$p < 0.01$). WBC: white blood cells; LCS: Leukocytospermia.

ANDROLOGY

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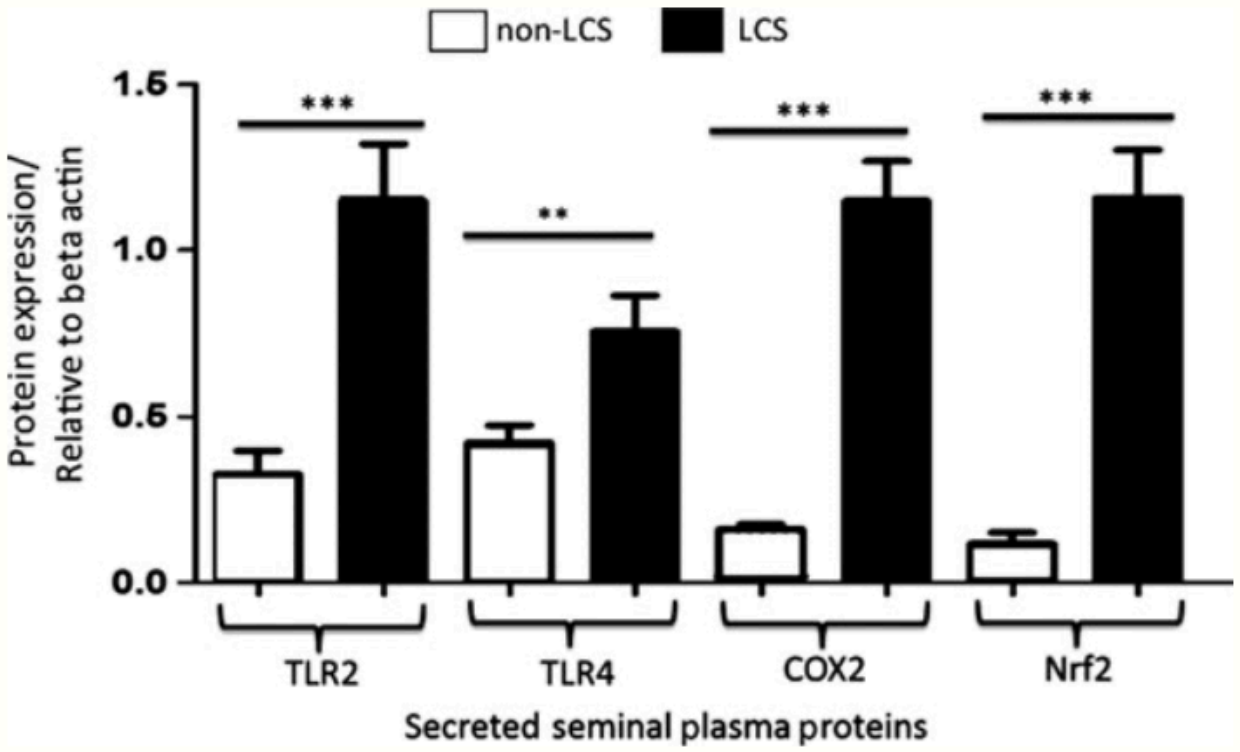


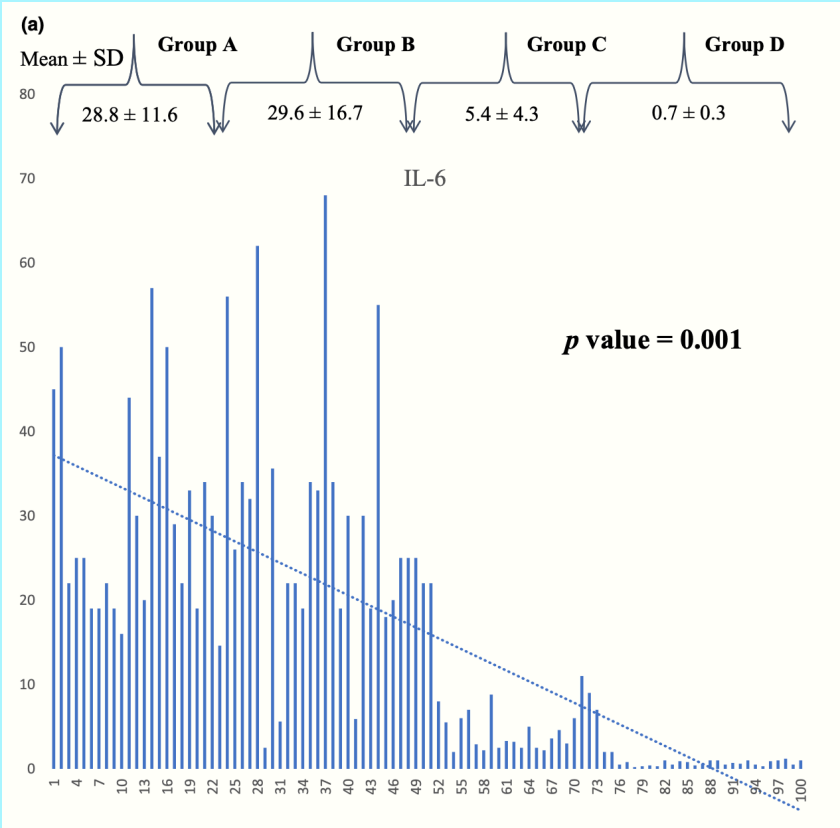
Figure 2 Western blot analysis of TLR-2, TLR-4, COX-2, and Nrf-2 expression in selected non-LCS and leukocytospermic (LCS) seminal plasma extracts. Non-LCS ($n = 15$) seminal plasma display a lower expression of TLR-2, TLR-4, COX-2, and Nrf-2 compared with LCS ($n = 15$) seminal plasma extracts. Data expressed as relative optical density ratio of TLR-2, TLR-4, COX-2, and Nrf-2 to β-actin. Values are mean ± SEM; significant differences (***$p < 0.001$ and **$p < 0.01$).

ANDROLOGY

Association between leukocytospermia and semen interleukin-6 and tumor necrosis factor-alpha in infertile men

Andrology, 2018, 6, 775–780

E. M. Eldamnhoury, G. A. Elatrash, H. M. Rashwan and A. I. El-Sakka
Department of Urology, Suez Canal University, Ismailia, Egypt



Leucocitospermia associa-se a uma *upregulation* da IL-6 e TNF-alfa seminais

A. Inférteis com leucocitospermia e parâmetros seminais normais
B. Inférteis com leucocitospermia e parâmetros seminais anormais
C. Inférteis sem leucocitospermia e parâmetros seminais anormais
D. Férteis sem leucocitospermia e parâmetros seminais normais

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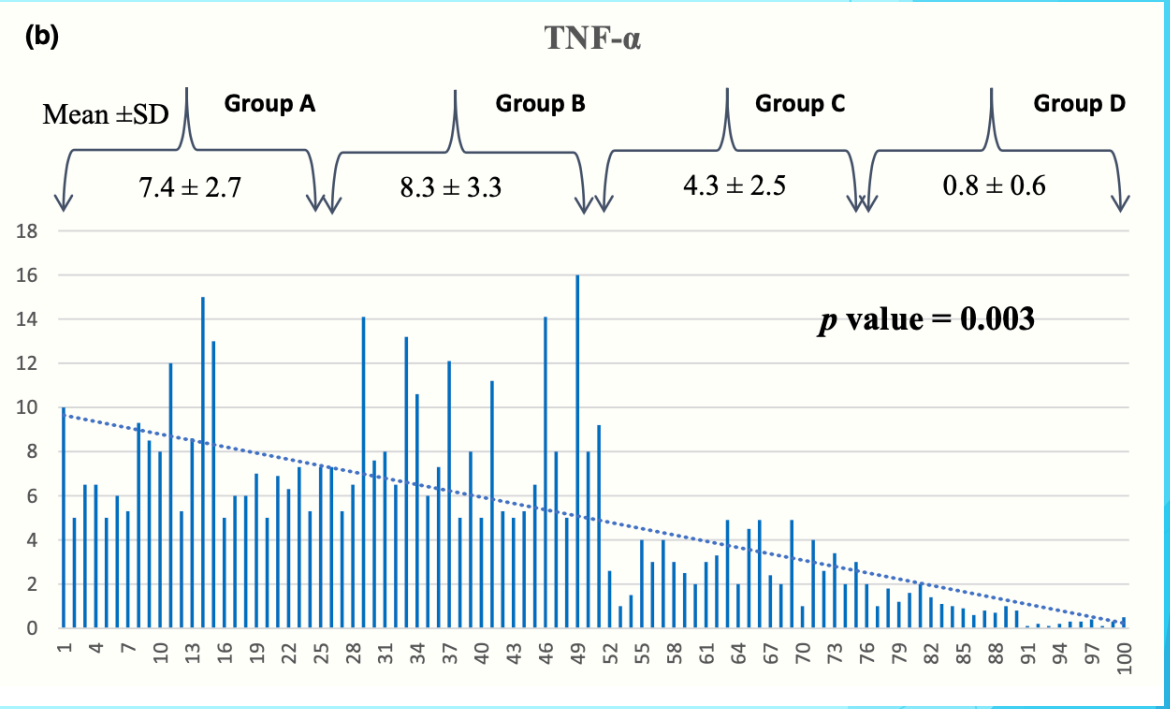

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D. Férteis sem leucocitospermia e parâmetros seminais normais

SPECIAL ISSUE

WILEY AJRI American Journal of Reproductive Immunology

Identification of seminal markers of male accessory gland inflammation: From molecules to proteome

Giuseppe Grande[1,2] | Domenico Milardi[1,2] | Silvia Baroni[3] | Giovanni Luca[4] | Alfredo Pontecorvi[2]

Am J Reprod Immunol. 2018;e12992.
https://doi.org/10.1111/aji.12992

TABLE 1 Advantages and disadvantages of the semen markers of MAGI and relation with semen parameters

Marker	Advantages	Disadvatages	Correlation with semen parameters
Leukocytospermia	Correlation with poor semen parameters	Not present in all cases of inflammation; difficulties in differentiating leukocytes from immature germ cells[14,15]	Strong correlation with semen parameters for seminal granulocyte concentration >2 × 10^6/ml[62]
Polymorphonuclear elastase	Previously reported to have stronger discrimination power for MAGI than leukocytospermia.[63] Defined cut-off in a previous study (230 ng/mL)[16]	Not used as routine determination; concentrations of PMN elastase not significantly related to biochemical parameters, to local anti-sperm antibodies and to the outcome of the microbial screening[17]	No correlation observed with seminal parameters and with sperm functional capacity[17]
α-glucosidase	Reduced in epidydimitis[21]	No recent studies, reporting correlation with other MAGI markers. No cut-off values	Correlation with reactive oxygen species markers
Citric acid	Reduced in prostatitis[20,64]	The correlation with seminal parameters is debated[65]	Correlation with semen volume
c-Glutamyl-trasnpeptidase	Reduced in prostatis. Correlation with the number of bacteria per ml[66]	Only few studies. No cut-off values	Correlation with semen volume
Zinc	Presence of recent meta-analysis studies confirming the reduction in chronic prostatitis.[67] Correlation with serum free testosterone.[68] Reduced in infertile males[69]	Isolated studies not confirming the correlation with prostatitis[70]	Correlation with sperm motility, morphology, viability[71] and function[72]
IL-6	Several studies reporting an increase in IL-6 in chronic prostatitis and in asymptomatic prostatitis[26,27,29] Correlation with leukocyte count.[67]Decreased after antibiotic treatment[28]	No standardized cut-off values	No correlation with semen parameters[73]
IL-8	Increased in prostatitis. Several studies[26,30,32,33,.] Correlation with leucocyte count.[12] Correlation with ultrasound signs of MAGI[12]	No standardized cut-off values	Correlation with semen volume
TNF-α	Correlation with leukocyte count[36]and Il-1β.[37] Increased in prostatitis[38]	Only few studies. No cut-off values	Only partial detrimental effects on sperm motility and membrane integrity[74]
IL-1β	Few papers reporting and increase in prostatitis[38,38,39]	Only few studies. No correlation with seminal parameters, with the outcome of the microbial screening and with male fertility[75]	No correlation with semen parameters[75]
HSP70	Increased in HSP70 in prostatitis. Correlation with symptom score in patients with chronic bacterial prostatitis and CP/CPPS	Only 1 paper[39] reporting this evidence	No data about correlation with semen parameters
IL-10	Reduced in prostatitis after antibiotic treatment. Proposed as marker of effectiveness of antibiotic treatment in patients with prostatitis[42]	Only few studies. No cut-off values	No data about correlation with semen parameters
Tool-like receptors 2 and 4	Increased in prostatitis. Correlation with oxidative stress	Only 1 paper reporting this evidence	No data about correlation with semen parameters
COX-2			
Nrf-2			Correlation with sperm motility and specific sperm function parameters[76]
suPAR	Increased in MAGI, independently by the origin (bacterial/abacterial). Presence of a cut-off value.[13] Reduced in hypogonadism.[57] Increased in patients with systemic inflammation (psoriasis)[56]	Only few studies	Correlation with sperm parameters

Central European Journal of Urology

Cent European J Urol. 2018; 71: 470-475

ORIGINAL PAPER

ANDROLOGY AND SEXUAL UROLOGY

Impact of leukocytospermia on sperm dynamic motility parameters, DNA and chromosomal integrity

Alaa Moubasher[1], Heba Sayed[1], Eman Mosaad[2], Ahmed Mahmoud[3], Fawzy Farag[4], Emad A. Taha[1]

[1]Department of Dermatology, Venereology and Andrology, Faculty of Medicine, Assiut University, Egypt
[2]Department of Clinical Pathology – South Egypt Cancer Institute, Assiut University, Egypt
[3]Department of Endocrinology and Andrology, Ghent University Hospital, Ghent, Belgium
[4]Department of Urology, Sohag University Hospital, Egypt

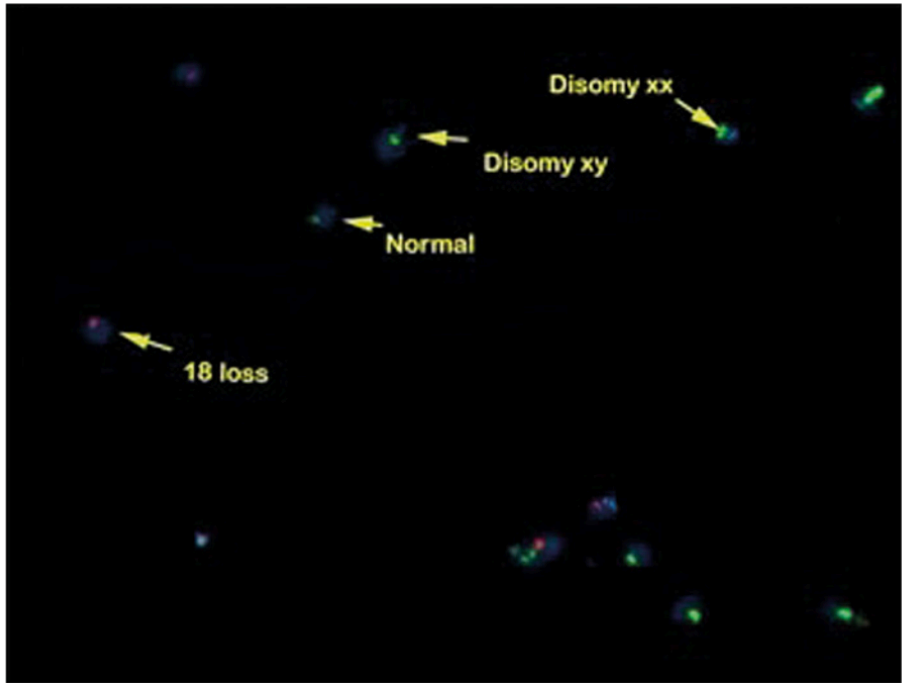

Figure 1. *Disomy XY, XX and 18 chromosome nullisomy by FISH in a case of leukocytospermia.*

Table 3. *Comparison of sperm DNA and chromosomal integrity between the two groups*

Semen variable	Leukocytospermia group	Non leukocytospermia group	*P*-value
Sperm DFI (%)			
Range	25–70	20–35	
Mean ±SD	43.60 ±13.43	30.20 ±5.10	<0.001
Disomy XY (%)			
Range	0–0.1	0–0.05	
Mean ±SD	0.017 ±0.027	0.004 ±0.012	<0.05
Disomy XX (%)			
Range	0–0.1	30–70	
Mean ±SD	0.009 ±0.025	50.1 ±9.68	>0.05
Disomy YY (%)			
Range	0–0.02	0–0.02	
Mean ±SD	0.002 ± 0.005	0.001 ±0.004	>0.05
Disomy 18 (%)			
Range	0–0.2	0–0.05	
Mean ±SD	0.032 ±0.048	0.005 ±0.011	<0.05
Percentages of semen samples with disomy (%)	68% (17/25)	36% (9/25)	<0.05

LEUCOCITOSPERMIA

Table 1. Study characteristics of randomized controlled trials of antibiotics for the treatment of leukocytospermia in male infertility

First author (year)	Population (n)	Leukocyte assessment method	Intervention (n)	Doses per day (mg, duration: day)
Baker (1984) [10]	Asthenospermic men (78)[a]	NR	A. Erythromycin[a] B. Placebo[a]	A. 250 (30)
Comhaire (1986) [11]	Infertile men with male accessory gland infection (33)	Peroxidase stain	A. Doxycycline (20) B. Placebo (13)	A. 100 (30)
Branigan (1994) [12]	Infertile men with prostatitis (102)	Monoclonal antibody HLe-1 staining	A. Doxycycline (25) B. Frequent ejaculation (25) C. Doxycycline with frequent ejaculation (25) D. No treatment (27)	A. 200 (7)+100 (21) B. At least every 3 days C. A+B
Merino (1995) [13]	Asymptomatic infertile men (60)	Papanicolaou stain	A. Trimethoprim/sulfamethoxazole (20) B. Ciprofloxacin (20) C. Placebo (20)	A. 320/1,600 (30) B. 500 (30)
Yanushpolsky (1995) [15]	Asymptomatic subfertile men (44)	Endtz test[b]	A. Doxycycline (18) B. Trimethoprim/sulfamethoxazole (18) C. No treatment (18)	A. 200 (14) B. 1,600 (14)
Yamamoto (1995) [14]	Infertile men with prostatitis (48)	NR	A. Trimethoprim/sulfamethoxazole (16) B. Trimethoprim/sulfamethoxazole with frequent ejaculation (17) C. No treatment (15)	A. 160/800 (30) B. 160/800 (30) with ejaculation (at least every 3 days)
Lewis-Jones (1996) [16]	Asymptomatic subfertile men (78)	Identification of round cells	A. Co-trimoxazole (31) B. Tetracycline (19) C. Trimethoprim (28)	A. 1,920 (90) B. 500 (90) C. 400 (90)
Erel (1997) [17]	Infertile men (70)	Peroxidase stain	A. Doxycycline (25) B. Doxycycline with ceftriaxone (21) C. Placebo (24)	A. 200 (10) B. 200 (10) with 1 g IM (1st day of the therapy)
Carranza-Lira (1998) [18]	Asthenozoospermic subfertile men (13)	NR	A. Ciprofloxacin (7) B. Trimethoprim/sulfamethoxazole (6)	A. 500 (14) B. 320/1,600 (14)
Vicari (2000) [19]	Infertile men with male accessory gland infection (122)	Immunocytochemical stain (WHO guideline 1992)	A. Ofloxacin or doxycycline (85) B. No treatment (37)	A. 400 (42)[c] or 100 (42)[c]
Krisp (2003) [20]	Asymptomatic patients with infertility or vasectomy (36)	NR	A. Levofloxacin (18) B. No treatment (18)	A. 250 (10)

NR: not reported, WHO: World Health Organization, IM: intramuscular injection, A, B, C, D: study group according to intervention.
[a]Cross-over study. Seventy-eight patients were randomized (35 had erythromycin then placebo, 5 erythromycin alone, 30 placebo then erythromycin, 8 placebo only).
[b]Reference from Endtz AW (Acta Cytol 1974;18:2-7) [21]. [c]Forty days each month for a 3-month period.

pISSN: 2287-4208 / eISSN: 2287-4690
World J Mens Health 2016 December 34(3): 165-172
https://doi.org/10.5534/wjmh.2016.34.3.165

Review Article

Treatment of Leukocytospermia in Male Infertility: A Systematic Review

Jae Hung Jung[1,2], Myung Ha Kim[2,3], Jiye Kim[2,4], Soon Koo Baik[2,5], Sang-Baek Koh[2,6], Hyun Jun Park[7], Ju Tae Seo[8]

https://doi.org/10.5534/wjmh.2016.34.3.165

[1]Department of Urology, [2]Institute of Evidence Based Medicine, [3]Yonsei Wonju Medical Library, [4]Department of Plastic Surgery, [5]Division of Gastroenterology and Hepatology, Department of Internal Medicine, [6]Department of Preventive Medicine, Yonsei University Wonju College of Medicine, Wonju, [7]Department of Urology, Pusan National University School of Medicine, Busan, [8]Department of Urology, Cheil General Hospital and Women's Healthcare Center, Dankook University College of Medicine, Seoul, Korea

LEUCOCITOSPERMIA

Table 2. Main outcomes and adverse events of randomized controlled trials of antibiotics for the treatment of leukocytospermia in male infertility

First author (Year)	Outcomes (follow-up: day)	Main results	Adverse events
Backer (1984) [10]	Sperm parameters (30)	Significant improvement of sperm motility	NR
Comhaire (1986) [11]	1) Sperm parameters (30, 60, 180) 2) Leukocytospermia (30, 60, 180) 3) Semen culture (30, 60, 180) 4) Pregnancy	1) Significant improvement of sperm motility 2) Significant improvement of leukocytospermia 3) Significant decrease of seminal bacteria 4) 2/20 in group A (1 in 5th and 1 in 7th month after intervention) *vs.* 1/13 in group B (5th month after intervention)	NR
Branigan (1994) [12]	1) Leukocytospermia (30, 90) 2) Pregnancy (90)	1) Significant improvement in all treatment groups at 1 Only group C had significant improvement at 3 months 2) 10 pregnancy (2 in A, 3 in B, 4 in C, 1 in D)	NR
Merino (1995) [13]	Sperm parameters (30)	Significant difference in semen pH in group A	None
Yanushpolsky (1995) [15]	1) Sperm parameters (30) 2) Leukocytospermia (30)	1) NS 2) NS	A. Nausea/diarrhea (2) B. Hypersensitivity (2), gastrointestinal upset (1)
Yamamoto (1995) [14]	1) Leukocytospermia (30, 60, 90) 2) Pregnancy (90)	1) Significantly greater resolution of leukocytospermia in treated than untreated group 2) 5/33 (treated) *vs.* 1/15 (untreated)	NR
Lewis-Jones (1996) [16]	1) Sperm parameters (90) 2) Leukocytospermia (90)	1) Significant improvement of sperm motility, morphology 2) Significant decrease of leukocyte count	NR
Erel (1997) [17]	1) Sperm parameters (30) 2) Leukocytospermia (30)	1) NS in sperm parameters 2) No significant reduction in leukocyte concentration	NR
Carranza-Lira (1998) [18]	Sperm parameters (74)	Morphology: decrease in the number of normal sperm in group A	NR
Vicari (2000) [19]	1) Sperm parameters (30, 90, 180) 2) Leukocytospermia (30, 90, 180) 3) ROS production (30, 90, 180) 4) Semen culture (30, 90, 180) 5) Pregnancy (180)	1) Significant improvement on concentration and motility 2) Significant resolution of leukocytospermia 3) Significant reduction in treated group 4) Significant improvement on bacteriologic cure 5) Significant improvement (24/85 [treated] *vs.* 2/37 [untreated])	A and B. dizziness, headache, nausea, vomiting, abdominal pain, or diarrhea (10)
Krisp (2003) [20]	1) Sperm parameters (30) 2) Leukocytospermia (30) 3) Semen culture (30)	1) NS 2) NS 3) Significant decrease of seminal bacteria	None

A,B,C,D: study group according to intervention (A~D of in this Table relate to the Table 1).
ROS: reactive oxygen species, NS: not significant, NR: not reported.

pISSN: 2287-4208 / eISSN: 2287-4690
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- Antibioterapia
  - Melhora os parâmetros seminais
  - Aumenta a taxa de resolução da leucocitospermia
  - Incrementa a taxa de cura bacteriológica
  - Aumenta a taxa de gravidez

- Anti-Oxidantes
  - Tendência a melhorar os parâmetros espermáticos "in vivo" mas os dados actuais são insuficientes

FERTILITY AND STERILITY
Vol. 62, No. 3, September 1994
Copyright © 1994 The American Fertility Society
Printed on acid-free paper in U. S. A.

Efficacy of treatment and recurrence rate of leukocytospermia in infertile men with prostatitis*

Emmett F. Branigan, M.D.†
Charles H. Muller, Ph.D.‡

Table 1 Effectiveness of Treatment at 1 Month

Treatment group	No. of subjects	Resolution of leukocytospermia	*P*
1 No treatment	25	1 of 25 (4)*	
2 Antibiotics alone	25	10 of 25 (40)†	<0.002
3 Frequent ejaculation alone	25	8 of 25 (32)†	<0.02
4 Antibiotic and frequent ejaculation	27	17 of 25 (68)†	<0.0001

* Values in parentheses are percentages.
† Significant differences when compared by χ^2 analysis with no treatment group.

Table 2 Effectiveness of Treatment at 2 and 3 Months

Treatment group	No. of subjects	Resolution of leukocytospermia	
		2 months	3 months
1 No treatment	24	2/24 (5)*	2/24 (5)
2 Antibiotics alone	23	4/23 (17)	4/23 (17)
3 Frequent ejaculation alone	22	3/22 (13)	2/22 (9)
4 Antibiotic and frequent ejaculation	23	13/23 (56)	12/23 (55)†

* Values in parentheses are percentages.
† Significantly different ($P = 0.007$) when compared by χ^2 analysis with groups 1, 2, and 3.

Leucocitospermia e cultura negativa

Como eu faço

Não peço cultura; repito espermograma em 30 dias

Elevado índice resolução espontânea

Azitromicina 1,0g dose única (casal)+ ejaculações frequentes

10-20% infecções bacterianas subclínicas

Antioxidantes (Vit C 500mg + Vit E 400mg + ácido fólico 5mg + Complexo B + Zinco 7mg + Selênio 20mcg) 3 meses

Diminuição estresse oxidativo

Esteves, 2010

Sandro Esteves, 2010

Cavagna *et al. Reproductive Biology and Endocrinology* 2012, **10**:44
http://www.rbej.com/content/10/1/44

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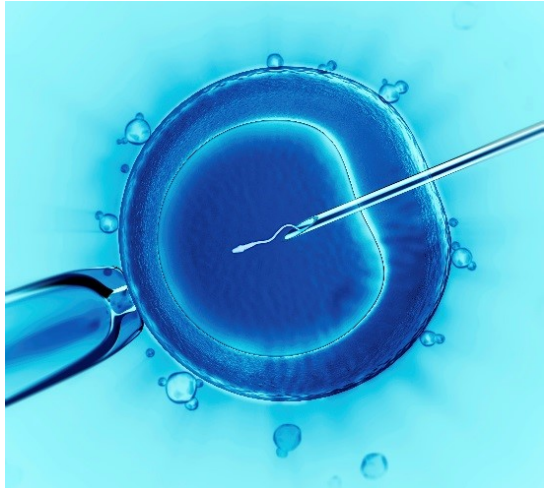


RESEARCH **Open Access**

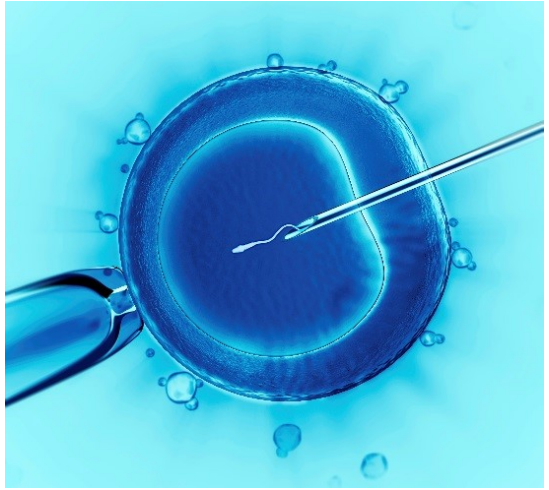
The influence of leukocytospermia on the outcomes of assisted reproductive technology

Mario Cavagna[1,2,3], Joao Batista A Oliveira[1,3,4], Claudia G Petersen[1,3,4], Ana L Mauri[1,3], Liliane F I Silva[1,3,4], Fabiana C Massaro[1,3], Ricardo L R Baruffi[1,3] and José G Franco Jr[1,3,4*]

Table 1 Comparison between the ICSI and IMSI procedures

	Total		*P*	Leukocytospermia		*P*	Non-leukocytospermia		*P*
	ICSI	IMSI		ICSI	IMSI		ICSI	IMSI	
Cycles	50	50		25	25		25	25	
Leukocytes in semen ($x10^6$)(mean ± SD)	1.5 ± 1.8 (0–8)	2.5 ± 7.5 (0–50)	0.41	2.4 ± 1.7 (1–8)	4.8 ± 10.5(1–50)	0.10	0.4 ± 0.3 (0–0.8)	0.4 ± 0.3 (0–0.8)	0.76
Female age (years)	34.4 ± 3.6	34.8 ± 3.9	0.67	34.4 ± 3.8	34.8 ± 4.1	0.75	34.5 ± 3.6	34.8 ± 3.8	0.79
Male age (years)	36.9 ± 5.0	37.2 ± 5.6	0.80	37.2 ± 5.0	37.4 ± 5.2	0.91	36.7 ± 5.0	37.0 ± 6.0	0.81
Fertilisation rate (%)	59.9 ± 28.7	63.5 ± 26.5	0.55	57.9 ± 30.2	67.6 ± 24.6	0.35	61.9 ± 27.7	59.5 ± 28.1	0.86
Embryos transfer (n)	2.1 ± 0.9	2.4 ± 0.8	0.21	2.2 ± 0.9	2.3 ± 0.8	0.90	2.1 ± 1.0	2.6 ± 0.9	0.10
Implantation rate (%)	13.2% (14/106)	17.2% (19/111)	0.54	12.3% (7/54)	17.5% (10/57)	0.68	13.5% (7/52)	16.7% (9/54)	0.84
Pregnancy/cycle (%)	24% (12/50)	26% (13/50)	0.81	24% (6/25)	28% (7/25)	0.74	24% (6/25)	24% (6/25)	1
Miscarriage (%)	0	7.7% (1/13)	0.32	0	14.3% (1/7)	0.33	0	0	——
Ongoing pregnancy/cycle (%)	24% (12/50)	24% (12/50)	1	24% (6/25)	24% (6/25)	1	24% (6/25)	24% (6/25)	1
Live birth/cycle (%)	24% (12/50)	24% (12/50)	1	24% (6/25)	24% (6/25)	1	24% (6/25)	24% (6/25)	1

Effect of seminal leukocytes on in vitro fertilization and intracytoplasmic sperm injection outcomes



Giuseppe Ricci, M.D.,[a,b] Marilena Granzotto, Ph.D.,[a] Stefania Luppi, Ph.D.,[a] Elena Giolo, Ph.D.,[a] Monica Martinelli, Ph.D.,[a] Gabriella Zito, M.D.,[b] and Massimo Borelli, Ph.D.[c]

[a] Institute for Maternal and Child Health, IRCCS "Burlo Garofolo," Trieste, Italy; and [b] Department of Medicine, Surgery, and Health Sciences and [c] Department of Life Science, University of Trieste, Trieste, Italy

Fertility and Sterility® Vol. 104, No. 1, July 2015 0015-0282/$36.00
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http://dx.doi.org/10.1016/j.fertnstert.2015.04.007

- A leucocitospermia não influencia negativamente os *outcomes* da FIV e ICSI
- A significância da leucocitospermia continua por estabelecer

Relationship between leukocytospermia, reproductive potential after assisted reproductive technology, and sperm parameters: a systematic review and meta-analysis of case–control studies

Andrology, 1–11

[1]C. Castellini, [1]S. D'Andrea, [1]A. Martorella, [1]E. Minaldi, [2]S. Necozione, [1]F. Francavilla, [1]S. Francavilla and [1]A. Barbonetti

[1]Andrology Unit, Department of Clinical Medicine, Life, Health and Environment Sciences, , and [2]Epidemiology Division, Department of Clinical Medicine, Life, Health and Environment Sciences, University of L'Aquila, L'Aquila, Italy

ANDROLOGY

CONCLUSÃO

1. Antibióticos e antioxidantes são o tratamento mais convencional
2. Não é possível estabelecer uma estratégia de tratamento universal
3. Ejaculações frequentes?
4. Parâmetros espermáticos e a resolução da leucocitospermia parecem ser melhores nos doentes submetidos a terapêutica
5. Mas apenas 1/4 ensaios demonstrou benefício da taxa de gravidez nos doentes submetidos a tratamento
6. Efeitos secundários ligeiros
7. Não retardar em demasia as técnicas de PMA
8. A leucocitospermia parece não exercer influência nos *outcomes* das técnicas de PMA

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