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EVALUATION OF SEMINAL PARAMETERS IN VARICOCELECTOMIZED MEN TREATED WITH *Lepidium meyenni* (Maca)

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ABSTRACT

Varicocele is the most frequent cause of male infertility which is characterized by poor seminal quality with high levels of reactive oxidative species. Surgical correction is considered the treatment of choice in this disorder and few studies focus on improving semen

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quality after varicocelectomy. In this study we determine the effect of *Lepidium meyenii* in patients with varicocele and post varicocelectomy. Semen samples were evaluated in thirty patients with infertility and varicocele grades II and III before surgery, at three and six months after surgery. At three months after surgery men were evaluated again and treated with 1,200 mg/day of *L. meyenii*. After three months post-surgery, an increase in sperm parameters was observed with a reduction in seminal viscosity; but at six months the improvement of the seminal parameters was more evident in treated men. Samples of patients with severe oligozoospermia improved after treatment with *L. meyenii*. Probably the antioxidant effect of polyphenols of maca are reducing factors of oxidative stress in the varicose testis. It is concluded that the administration of *L. meyenni* in patients with varicocele helps the restoration of sperm quality and probably in fertility.

Keywords: Varicocele, oxidative stress, male infertility, male accessory glands, *Lepidium meyenii*.

EVALUACIÓN DE LOS PARÁMETROS SEMINALES EN HOMBRES VARICOCELECTOMIZADOS TRATADOS CON *Lepidium meyenni* (Maca)

RESUMEN

El varicocele es la causa más frecuente de la infertilidad masculina que se caracteriza por una mala calidad seminal con altos niveles de especies reactivas del oxigeno. La corrección quirúrgica se considera el tratamiento de elección en este trastorno y escasos estudios se centran en mejorar la calidad del semen después de la varicocelectomía. En este estudio se determinó el efecto de *Lepidium meyenii* en pacientes con varicocele y post varicocelectomía.

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Se evaluaron muestras de semen en treinta pacientes infértiles con varicocele grados II y III antes de la cirugía, a los tres y seis meses después de la cirugía. Tres meses después de la cirugía, los hombres fueron evaluados nuevamente y tratados con 1.200 mg/día de *L. meyenii*. Después de tres meses, se observó un aumento en los parámetros de esperma con una reducción en la viscosidad seminal; pero a los seis meses la mejora de los parámetros seminales fue más evidente en los hombres tratados. Las muestras de pacientes con oligozoospermia grave mejoraron después del tratamiento con *L. meyenii*. Probablemente el efecto antioxidante de los polifenoles de la maca son factores reductores del estrés oxidativo en los testículos varicosos. Se concluye que la administración de *L. meyenni* en pacientes con varicocele ayuda a la restauración de la calidad del esperma y probablemente a la fertilidad.

Palabras clave: Varicocele, estrés oxidativo, hombre infértil, glándulas accesorias masculinas, *Lepidium meyenii.*

INTRODUCTION

Male infertility refers to a male's inability to result pregnancy in his couple. Male factor infertility is seen as an alteration in sperm density and/or motility and/or morphology in at least one sample of two sperm analyzes, collected 1 and 4 weeks apart (1). Varicocele is the most frequent cause into the factors that affect male fertility (35%) (2). It´s an abnormal dilatation, elongation and tortuosity of the pampiniform plexus veins of the spermatic cord, and is more frequent on the left side. Often seminal parameters are more altered in grades II- III that grades I-II of varicocele (3).

Different mechanisms have been proposed in the physiopathology of varicocele such as scrotal hyperthermia, hypoxia, hormonal imbalances and reflow of metabolites from renal and/or adrenal glands (2). These conditions are associated with imbalance between reactive oxygen species (ROS) and antioxidant protection, leading to oxidative stress, which can affect cell function through

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mechanisms such as lipid peroxidation of the sperm membrane and fragmentation of nuclear material of the sperm (4). Thus, in the characteristic pathological process of varicocele, male infertility and oxidative stress are closely linked (5). Although surgery is the most recommended therapeutic option to varicocele, the efficacy of varicocelectomy on seminal quality and on fertility has been a very controversial topic (6-9). Varicocelectomy has resulted in rapid significant decline in free radical levels and slow (3-6 months) (10).

The search for therapeutic alternatives has identified in traditional medicine the use of medicinal plants, including certain varieties of *Lepidium meyenii* (maca) that have been used since ancient times in the Alto-Andean culture for their effects on fertility. Previous work shows that the administration of *L. meyenii* in adult male rats produced an increase in the weight of testicles and epididymis, revitalizing spermatogenesis (11). Considering that oxidative stress is determinant in the decreasing of seminal quality in men with varicocele, the present study was conducted to determine the effect of *L. meyenii* (maca) in subjects with varicocele, after varicocelectomy and three months post-treatment after varicocelectomy according to clinical parameters and biomarkers of male accessory glands.

MATERIALS AND METHODS

A prospective study was carried out in two groups: a study group comprising 40 patients (age 24-45) with grades II or III of varicocele. Patients were attended in single center (CEDIEG) for andrological evaluation. All subjects were evaluated by the same infertility specialists (medical records, physical examination and inclusion/exclusion criteria) and a semen analysis was performed according to the WHO fifth edition (2010) guidelines for semen analysis (12). Exclusion criteria were: the presence of urogenital infections, azoospermia, hypogonadism and systemic diseases (cancer and endocrine pathology). The presence of a clinical varicocele was determined by palpation and observations in the standing position before and during the Valsalva manoeuvre and confirmed by colour Doppler ultrasonography. Varicocele was classified as grade II (palpable without Valsalva manoeuvre) or grade III (visible through the scrotal skin). Informed written consent was obtained as well as approval for

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the investigation project established by the Declaration of Helsinki for human research outlined in the Code of Bioethics and Biosafety FONACIT (Fondo Nacional de CienciaTecnología e Innovación) (13). Semen samples were collected by masturbation after 2-7 days of sexual abstinence and analyzed within 1 h of ejaculation. The semen volume, total motility, normal forms and sperm count were determined. Seminal plasma was separated by centrifugation of semen at 1200×g, and stored at −20 °C to until neutral alpha glucosidase, fructose and zinc measures as biomarkers of epididymis, seminal vesicles and prostate respectively (14) and viscosity by semicuantitative method was measured (15). Each semen sample (n=40) was analyzed before surgery (varicocele) and three months after surgery (3m-AS). Later six months after surgery 30 men with the last three months with treatment (3m-AS+3mT) and 10 men without treatment with *L. meyenii* (6m-AS). The administered product consisted of a micro pulverized of *L. meyenii* subjected to desiccation with warm air flow, variety "wajcha", called Maca Spirit™®, the same were commercial products of Laboratorios NATURALCOS S.A. After three months of surgery thirty patients were treated with 1,200 mg/day of maca and ten of them did not follow the treatment.

Statistical analysis was performed using SPSS 10.0 statistical software for Windows (SPSS, Chicago, IL, USA). Basic descriptive statistics (mean values ± SD) were calculated for the varicocele group and the control group. Differences between the varicocele and control groups were determined using ANOVA-test and a P value < 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

After varicocelectomy and after surgery + treatment with maca, the following changes were observed: a significant increase in sperm density and motility with a significant reduction of round cells and leukocytes. Sperm forms showed a greater increase in the normal forms when fulfilling the treatment with *L. meyenii* (Table 1).

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Table 1. Values of spermogram pre and post treatment measured along the study.

Characteristic	Varicocele (n=40)	3m-AS (n=40)	3m-AS +3mT (n=30)	6m-AS (n=10)	RV
Sperm density x10^6mL^{-1}	106.1±13.4	112.8±25.5	124.9±56.0 [a]	118.1±61.2	> 15
Sperm density/ejaculate	222.8±27.6	327.1±59.6 [b]	405.6±100.5[b,d]	253.5±121.1[c,f]	> 39
Total motility	27.9± 14.3	37.9±14.5 [b]	47.8± 15.8[b,d]	41.8± 13.9[b,d]	> 40
Sperm normal forms	15.8± 9.3	17.4± 10.1[ns]	20.1± 8.3 [a]	17.9± 11.8	> 4
Round cells/mL	5.5±3.3	4.0± 3.2 [a]	1.2 ±0.2[b,d]	4.0 ± 1.3 [f]	< 5
Leukocytes/mL	0.9± 0.5	0.6± 0.2[b,d]	0.2±0.2[b,d]	0.6±0.5[b,f]	<1

Groups varicocele, three months after surgery (3m-AS); six months after surgery with the last three months with treatment with *L. meyenii* (3m-AS+3mT) and six months after surgery without treatment (6m-AS). Comparisons of ach group with respect to varicocele [a]<0.05; [b]<0.005;3m-AS[c]p <0.05, [d]p<0.005; and 3m-AS+3mT [e]<0.05 [f]p<0.005. RV: Reference value.

The seminal volume increased progressively after surgery, the increase being more evident when the treatment with *L. meyenni* was fulfilled. Viscosity was reduced three months after surgery. Seminal vesicle marker (fructose) values showed no changes in any of the groups. The epididymal function marker (neutral alpha- glucosidase, NAG) showed a significant increase to the other groups in the patients who complied with the treatment with *L. meyenni* (Table 2).

Table 2. Values of volume, viscosity and biomarkers of male accessory glands determined pre and post treatment during the study.

Characteristic	Varicocele (n=40)	3m-AS (n=40)	3m-AS +3mT (n=30)	6m-AS (n=10)	RV
Volume (mL)	2.1± 1.3	2.9±1.4[a]	3.3±1.3[b]	2.8 ±1.5[b]	> 1.5
Viscosity (scale 0-4)	1.4±1.2	0.8± 0.8 [a]	0.5±0.1 [b]	0.6±0.7 [b]	≤1
Fructose (µmol/ejaculate)	52.5± 31.9	53.8±27.3	52.8±33.2	49.8± 13.9	> 13
Zinc (µmol/ejaculate)	8.9 ± 7.6	14.4± 7.8[b]	13.1±8.2 [b]	13.9± 11.8[b]	> 2.4

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NAG (mU/ejaculate)	15.3± 6.4	17.0±8.9	22.3± 19.1[b,c]	17.1 ± 11.3[f]	> 20

Groups varicocele, three months after surgery (3m-AS), six months after surgery with the last three monthswith treatment (3m-AS+3mT) and six months after surgery without treatment with *L. meyenii* (6m-AS).Comparisons of ach group with respect to varicocele [a]<0.05; [b]<0.005,3M-AS[c]p <0.05, [d]p<0.005; and 3m-AS+3mT [e]<0.05 [f]p<0.005. NAG: neutral alpha-glucosidase. RV: Reference value.

Changes in sperm density increased after surgery. In those men who did not comply with the treatment (n = 10) there was a slight increase in sperm density and increased motility; nevertheless, the changes are more evident when the treatment was completed. The increase on motility and sperm density has been observed in another study without changes in gonadotropic hormone levels after 12 weeks of testing (16). Normal forms did not improve after three (n=40) and six months (n=10) of varicocelectomy, but after treatment with maca there was increase in the normal forms (n=30). It was demonstrated by Tancara *et al*. (17) in infertile men without varicocele treated with maca after 12 weeks of treatment. They found no improvement in sperm density with maca. Our results show an increase in seminal volume in men who received treatment with maca and an important increase in the number of sperm per total ejaculate with an insignificant increase in the number of sperm per milliliter like other study (18). In the present study the normal forms was more increased after treatment with maca. The characteristics density, motility, morphology have been considered to evaluate the fertility status of patients with varicocele. These parameters improved after surgery. Guzick *et al*. (19) considered subfertility due to semen characteristics <13.5 million mL^{-1}, motility <32%, and normal morphology <9%. They considered that the percentage of normal semen morphology was the most powerful

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discriminator between fertile and infertile populations.

The seminal viscosity is high and could be restored after the varicocelectomy. It is very likely that the reduction in viscosity is due to varicocelectomy and not to treatment with maca. Viscosity has been associated with prostatic dysfunction. It has been reported that in the presence of varicocele there is periprostatic vascular flow alterations which may decrease after surgery (20). An important characteristic into the varicose testis is the oxidative stress, so it is worth mentioning the components of the commercial *L. meyenii* that may favor the physiology of the male reproductive tract. Several groups of biologically active constituents have been identified in maca as aromatic glucosinolates (benzyl and p-methoxbenzylglucosinolates in particular) and their isothiocyanate derivatives (21). The hydroalcoholic extract powder of maca for 6 weeks increases serum testosterone concentration associated with seminal vesicle stimulation in male rats, and this increase in testosterone level may be related to the enhanced ability of testosterone production by Leydig cells especially in the metabolic process following cholesterol (22). Also, high antioxidant activity has been detected in the methanolic fraction than in the aqueous fraction of yellow or black maca (23). Polyphenols are antioxidant molecules that, like antioxidant vitamins and enzymes, help prevent the oxidative stress caused by excessive ROS. The antioxidant properties of polyphenols are primarily due to the presence of hydroxyl groups (24). The evaluation of compounds of the seminal plasma has been useful to understand the process of natural fertilization and to achieve pregnancy naturally when the causes of infertility in man have been clearly established (25).

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CONCLUSION

In conclusion, treatment with maca can help restore seminal quality, especially in sperm morphology and seminal volume where low oxidative stress and stimulation of Leydig cells may be involved.

REFERENCES

1. World Health Organization.WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction.4th ed. Cambridge: Cambridge University Press;1999, pp. 1-86.
2. Madgar I, Weissenberg R, Lunenfeld B, Karasik A, Goldwasser B. Controlled trial of high spermatic vein ligation for varicocele in infertile men. Fertility and Sterility. 1995, 63:120-124.
3. Vivas-Acevedo G, Lozano JR, Camejo MI. Effect of varicocele grade and age on seminal parameters. Urologia Internationalis. 2010, 85:194-199.
4. Vivas-Acevedo G, Lozano-Hernández R, Camejo MI. Varicocele decreases epididymal neutral α-glucosidase and is associated with alteration of nuclear DNA and plasma membrane in spermatozoa. British Journal of Urology International. 2014, 113(4):642-649. doi: 10.1111/bju.12523.
5. Miyaoka R, Esteves SC. A critical appraisal on the role of varicocele in male infertility. Advances in Urology. 2012, ID 597495, doi:10.1155/2012/59749597495.
6. Abdel-Meguid TA, Al-Sayyad A, Tayib A, Farsi HM. Does varicocele repair improve male infertility? An evidence-based perspective from a randomized, controlled trial. European Urology.2011,59:455-461.
7. Kroese AC, de Lange NM, Collins J, Evers JL. Surgery or embolization for varicoceles in subfertile men. The Cochrane database of systematic review.2012 10:CD000479.doi: 10.1002/14651858.
8. Baazeem A, Belzile E, Ciampi A, Dohle G, Jarvi K, Salonia A, Weidner W, Zini A. Varicocele and male factor infertility treatment: a new meta-analysis and review of the role of varicocele repair. Eurepan Urology. 2011,60:796-808.

Recibido: 25/0/2019
Aceptado:15/03/2019

9. Agarwal A, Deepinder F, Cocuzza M, Agarwal R, Short RA, Sabanegh E, Marmar JL. Efficacy of varicocelectomy in improving semen parameters: new meta-analytical approach. Urology.2007,70:532-538.
10. Dada R, Shamsi MB, Venkatesh S, Gupta NP, Kumar R. Attenuation of oxidative stress & DNA damage in varicocelectomy: Implications in infertility management. The Indian Journal of Medical Research.2010,132:728-730.
11. Gonzales GF, Ruiz A, Gonzales C, Villegas L, Cordova A. Effect of *Lepidium meyenii* (MACA) roots on spermatogenesis of male rats. Asian Journal of Andrology.2001. 3:231-233.
12. World Health Organization. Manual for the Examination and Processing of Human Semen, 5th edn. Geneva: WHO Press.2010.
13. Briceño E, Suárez E, Michelangi C, Feliciangeli D, Ptaiza E, Mendible J. Código de Bioética y Bioseguridad. Ministerio de Ciencia y Tecnología (FONACIT). 2ª edición. Venezuela. 2002.
14. Vivas-Acevedo G, Lozano-Hernandez R, Camejo MI. Markers of accessory sex glands function in men with varicocele, relationship with seminal parameters. Canadian Journal of Urology.2011,18:5884-5889.
15. Lozano-Hernández R, Gualdrón J, Nava D, Rojas-Lozano MA. Efecto de la hiperviscosidad seminal sobre la integridad acrosómica y la movilidad espermática antes y después de la criopreservación. Investigación Clínica [Internet]. 2016 Sep [citado 2018 Ene 06]; 57(3): 267-279. Disponible en: http://www.scielo.org.ve/scielo.php?script=sci_arttext&pid=S0535-51332016000300005&lng=es.
16. Melnikovova I, Fait T, Kolarova M, FernandezEC,LuigiMilella L. Effect of *Lepidium meyenii* Walp. on semen parameters and serum hormone levels in healthy adult men: A double-blind, randomized, placebo-controlled pilot study. Evidence-Based Complementary and Alternative Medicine.2015 ID 324369, doi.org/10.1155/2015/324369

Recibido: 25/0/2019
Aceptado:15/03/2019

17. Tancara MSEBQ, Cortez J, Velez G, Salcedo Y, Salinas AM, Carvajal R. Effect of the *Lepidium meyenii* (Maca) on the spermatogenesis and the spermatic quality of subjects with diagnosis of infertility: study of cases. BIOFARBO. 2011,18:61-70.
18. Gonzales GF, Cordova A, Vega K, Chung A, Villena A, Goñez C, Castillo S. *Lepidium meyenii* (MACA) improved semen parameters in adult men. Asian Journal of Andrology. 2011, 3:301-303.
19. Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C, Carson SA, Cisneros P, Steinkampf MP, Hill JA, Xu D, Vogel DL. National Cooperative Reproductive Medicine Network. Sperm morphology, motility, and concentration in fertile and infertile men.The New England Journal of Medicine.2001,345:1388-1393.
20. Condorelli RA, Calogero AE, Mongioi' L, Vicari E, Russo GI, Lanzafame F, La Vignera SJ. Varicocele and concomitant dilation of the periprostatic venous plexus: effects on semen viscosity sperm parameters. Journal of Endocrinological Investigation. 2016, 39:543-547. doi: 10.1007/s40618-015-0401-9. Epub 2015 Oct 30.
21. Meissner HO, Mscisz A, Reich-Bilinska H, Kapczynski W, Mrozikiewicz P, Bobkiewicz-Kozlowska T, Kedzia B, Lowicka A, Barchia I . Hormone-Balancing effect of pre-gelatinized organic maca(*Lepidium peruvianum* Chacon): (II) physiological and symptomatic responses of early-postmenopausal women to standardized doses of maca in double blind, randomized, placebo-controlled, multi-centre clinical study. International Journal of Biomedical Science. 2011, 2:360-374.
22. Ohta Y, Yoshida K, Kamiya S, Kawate N, Takahashi M, Inaba T, Hatoya S, Morii H, Takahashi K, Ito M, Ogawa H, Tamada H. Feeding hydroalcoholic extract powder of *Lepidium meyenii* (maca) increases serum testosterone concentration and enhances steroidogenic ability of Leydig cells in male rats. Andrologia. 2016, 347-354. doi: 10.1111/and.12453. Epub 2015 Jul 14.
23. Inoue N, Farfan C, Gonzales GF. Effect of butanolic fraction of yellow and black

Recibido: 25/0/2019
Aceptado:15/03/2019

maca (*Lepidium meyenii*) on the sperm count of adult mice. Andrologia.2016**,** 48:915-921. doi: 10.1111/and.12679.

24. Verschooten L, Claerhout S, Van Laethem A, Agostinis P, Garmyn M. New strategies of photoprotection. Photochemistry and Photobiology.2006,82:1016-1023.
25. Lozano R. Male accessory glands and sperm function. IntechOpen 2018: 101-116. Disponible en: http://dx.doi.org/10.5772/intechopen.74321

Recibido: 25/0/2019
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