المؤتمر العلمي الدولي الاول للتقنيات الطبية والبيولوجية / للفترة 8-9 آذار 2017
Al-Kufa University Journal for Biology
Print ISSN: 2073-8854 & Online ISSN: 2311-6544

Serum Anti-Mullerian Hormone and Follicle Stimulating Hormone,Luteinizing hormone Concentrations in Infertile Iraqi men

Areej Shakeer Jassum and Dr.Kadhem Mohammed

Muthanna University College of Education of pure science Muthanna University College of Science

ABSTRACT

The objective of this study was to investigate the relationships betweenAnti-Mullerian hormone AMH and male infertility, which is defined as the inability of a couple to achieve pregnancy over the average period of one year of unprotected sexual intercourse. The study was conducted in Infertility Center of Al-Sader Teaching Hospital of AL-Najaf province, for the period from October 2014 until March 2015. The study included 70 blood samples from infertile men and 50 blood samples from their natural men were confirmed their fertility and have at least one child properly, has been considered as a group control. The ages of patients and the control group ranged between (20-50) years old. This study was conducted to determine the impact of AMH on male infertility, and find a relationship between the (AMH) and the level of the follicle stimulating hormone (FSH), luteinizing hormone (LH). Results were analyzed statistically between the control groups and those who suffer from infertility using SPSS12 system show significant differences when they exceed 95% ($P < 0.05$). There was a significant decrease in the level of (AMH) while an increase in the level of (FSH) and (LH) in patients group. The present study concludes that there is significant reduction in serum AMH of infertile men as compare with fertile men. However, there is negative correlation between AMH & FSH, LH serum in infertile men.

Introduction

Male Infertility define as primarily infertile if they have been incapable to get a pregnancy after one year of unprotected intercourse. Another define as failure to achieve a pregnancy within one year of normal, unprotected sexual intercourse(1)The WHO and the American Society for Reproduction Medicine Practice Committee defines infertility as after at least 12 months no pregnancy of unprotected sexual intercourse. Infertility becomes health trouble according to the WHO when the percentage exceeds about 1%(2,) . There for, it is projected that (8-12 % of couples) incident some form of infertility in their livers (3) . Because of the large differences in prevalence's of infertility among countries that might be due to differences in definitions and epidemiological designs (4).

Anti-Mullerian hormone (AMH)

Anti-Mullerian hormone (AMH) is a member of the transforming growth factor (TGF) super family (,5). It is also known as mullerian inhibiting substance (6). It's a peptide homo dimer of molecular weight 140 kDa that consists of two identical glycoprotein subunit, which is connected by disulphide bridge (*7*), which acts on tissue growth and differentiation. . It was identified as a factor which is being synthesized by

Al-Kufa University Journal for Biology
Print ISSN: 2073-8854 & Online ISSN: 2311-6544

testicular Sertoli cells, induces regression of the Mullerian ducts during male fetal development (*8) (* 9). Target organ for AMH in male are mullerian ducts and gonads for both sexes. In male the AMH is strongly expressed in Sertoli cell from testicular differentiation up to puberty and to a much lesser degree in granulose cells from birth up to menopause;(10) (11) (12) .

Follicle-Stimulating Hormone (FSH)

FSH is a 30 kDa hetero dimeric glycoprotein that belongs to a class of proteins that includes luteinizing hormone (LH), thyroid stimulating hormone, and human chorionic gonadotropin. Structurally, these glycol proteins consisting of a common (a) subunit and a hormone specific (b) subunit (13) Each of the subunits are encoded by single, separate genes (14)share a common alpha subunit, but have unique beta subunits that confer receptor specificity(15). The glycoprotein hormones act through specific G-protein coupled receptors (GPCRs) on target cell surfaces(16) (17)(18). FSH are key hormones in the endocrine control of vertebrate reproduction.`

In mammals follicle-stimulating hormone (FSH) is central to reproduction. On the surface of target cells it acts during a G-protein coupled receptor to stimulate testicular and ovarian functions. FSH is secreted from the pituitary gland to regulate reproduction in mammal(16)(19).

FSH binds to the FSH receptor, which belongs to the G-protein coupled super family characterized by their 7 hydrophobic trans membrane domains comprising intracellular and extracellular helices. The FSH receptor is coupled to the Gs subtype, which activates cyclic AMP (cAMP) when the receptor is activated by FSH(19) (20). In females, FSH targets a receptor (FSHR) expressed only on granulose cells, and induces the maturation of ovarian follicles(21). In males, FSH stimulates sertoli cell proliferation in testes and supports spermatogenesis(16) (22). FSH is used clinically to treat an ovulatory women and infertile men. The failure of pituitary to secret FSH and LH will result in disruption of testicular function leading to infertility.

Luteinizing hormone

LH is glycoprotein hormone which consist of two polypeptide chains and bear carbohydrate moieties N-linked to asparagines (As) residues , although the sub unit B is structurally very analogous , and differs in each hormone and confers specificity of action , the subunit is common to all members of the glycoprotein hormone family (23) .LH is secreted from the pituitary gland that control development , maturation and function of the gonad (24) .After the synthesis process is completed ,LH is stored in different secretion granules ,readily to be released upon stimulation with GnRH (25). Apposite function of LH is necessary for ovulation to occur in females and for Leydig cell development are necessary for the maintenance of early pregnancy by maintaining elevated levels of progesterone (17)

Al-Kufa University Journal for Biology
Print ISSN: 2073-8854 & Online ISSN: 2311-6544

Materials and Methods

This cross-sectional study was performed at the Infertility Center of Al-Sader Teaching Hospital of AL-Najaf province from November, 2014 to March, 2015. We assessed serum AMH and FSH levels and LH level on 70 infertile men and 50 healthy controls, who were admitted to infertility clinic to investigate infertility. The Patients according to age divided in to three groups(20-30, 31-40, 41-50)years and the range between (20- 50) years. Venous blood sample (5 ml) was collected from each male of both infertile and healthy control. The serum obtained by putting the blood samples in a clean dry plain plastic tube and allowed to clot at 37C for 30 minutes before centrifugation. The tubes centrifuged at 3000 rpm for 10 minutes, serum was collected and stored in freezer at -20 º C until used for estimation of the hormonal parameters. ELISA kit method using a kit provided by Beckman Couter, Inc USA. was utilized to measure Anti-Mullerian hormone (AMH) in both groups(26), while (FSH) and (LH) were determined by the Enzyme Linked Fluorescent Assay (ELFA) technique using a kit provided by Biomerieux, Inc Franc with minividas instrument.

Result

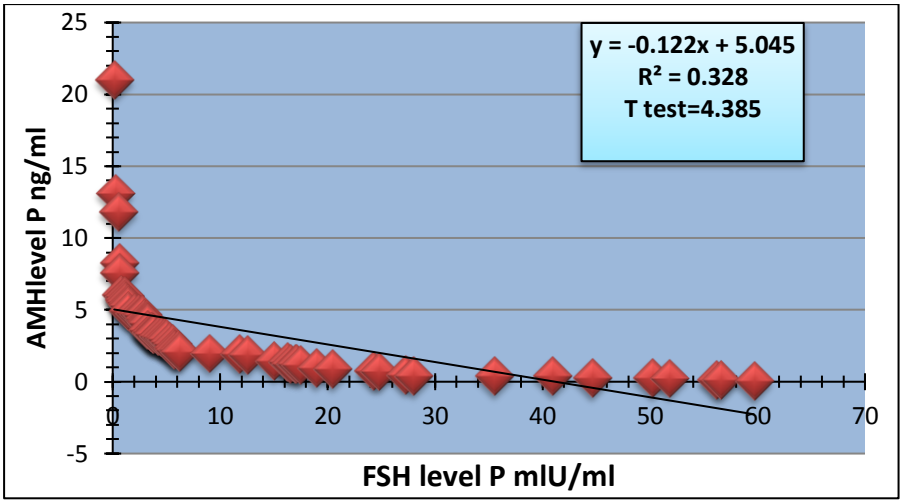
The laboratory values of hormones were significantly different in infertile men as compared to normal men as shown in table (1). That serum AMH concentrations and AMH concentrations were significantly lower in infertile men as compared to normal men. While, serum FSH concentrations and LH were found to be significantly higher than controls. In the figure (1) There was a significant negative correlations between AMH and FSH ($r= -0.572$, $p\leq0.0001$) in infertile men. Also There was a significant negative correlations between AMH and LH ($r=- 0.512$, $p\leq0.001$) in infertile men Figure (2) shows that serum

Table (1) : The means of AMH, FSH,LH in infertile and fertile men

Groups / Criteria	Mean ±S.E.			
	Fertile N=50	Patients N=70	T .test	P value
AMH ng/ml	6.3256±0.9162	3.4742±0.4466 *	2.80	0.083
LH mlU/ml	4.5724±0.7589	8.1546±1.3553 *	-2.31	0.0236
FSH mlU/ml	8.3648±2.0639	12.993±2.078 *	-1.58	0.1187

(*)Statistically significant differences ($p\leq 0.05$) between patients and fertile groups.

Al-Kufa University Journal for Biology
Print ISSN: 2073-8854 & Online ISSN: 2311-6544



Figure(1) Correlation between AMH and FSH in infertile men

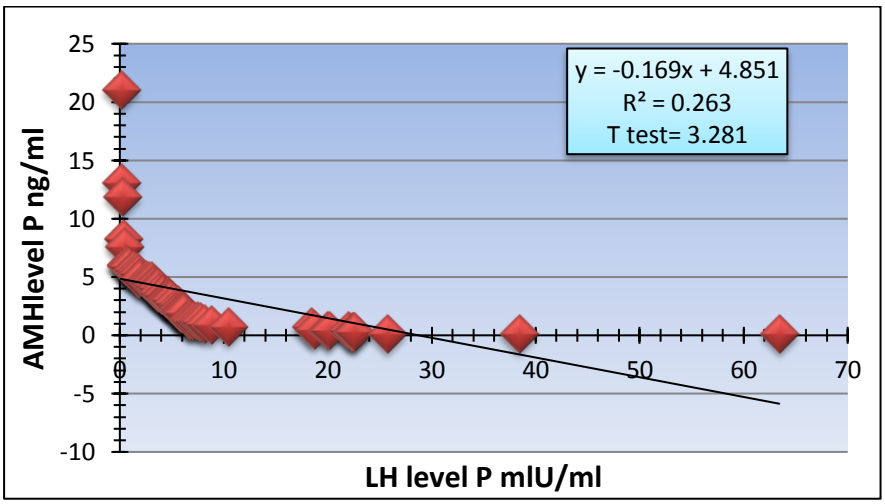


Figure (2) Correlation between AMH and LH in infertile men

Discussion

The present study shows a high level of FSH in patient. FSH evaluation is useful in the running of male infertility (26), and FSH obligatory for the initiation of spermatogenesis and maturation of spermatozoa. In infertile men, higher concentration of FSH is considered to be a dependable indicator of germinal epithelial harm (27). The rise in the levels of gonadotropins might disrupts the spermatogenic process leading to the decline in the sperm count and infertility(25).

The current study shows a significant negative correlation between AMH and FSH levels. That means AMH declines with the evaluation in FSH level. The result of present study may be due to reflect an involvement in the signaling and regulation of FSH or most probably to be a symptom of impaired or immature Sertoli cells (28). The present study is agreement with previous studies that reported negative correlation between AMH and FSH (29) (30). Other study suggested that AMH has no exact role in the regulation of FSH, Nor

Al-Kufa University Journal for Biology
Print ISSN: 2073-8854 & Online ISSN: 2311-6544

is AMH famous to inhibit FSH secretion or to be stimulated by gonadotropins (31). But disagreement with study that reported FSH is responsible for the abnormal elevation of serum AMH observed in the infertile patients,the effect of FSH on *testicular* production of AMH might lead to a direct effect on expression of AMH in each individual Sertoli cell, a proliferative effect on Sertoli cells (32). FSH receptors express on the Sertoli cells, and many Sertoli cell are stimulated by FSH to secretory products during pubertal development and in adult life (33). In patients, deficient androgen activity within the testis, FSH is capable of rising AMH secretion by Sertoli cells. Therefore, FSH was probable to be the gonadotropin accountable for AMH stimulation (34).

Sertoli cells generate and secrete inhibin B, a protein hormone which exerts on feedback affects the anterior pituitary to inhibit FSH secretion (35). Basal levels of AMH are independent of gonadotropin regulation, to The regulate of AMH after birth is complex, during childhood and in patients with hypogonadotropic hypogonadism (30). The negative correlations between serum levels of FSH and serum AMH concentrations might reveal an significant interest of AMH in signal and regulation of gonadotropin hormones and so the reproductive male system (36).

Experimental studies in mice clearly show that FSH increases testicular AMH production because of both Sertoli cell proliferation and enhancement of AMH expression in individual Sertoli cells (37). Earlier studies showed different results regarding the role of FSH on AMH production, one of the study show that FSH given to new born rats decreased AMH mRNA and immune reactive protein levels in the testis(38). While other study showed that increased levels of AMH mRNA in response to FSH when they cultured fetal Sertoli cells (39). AMH levels in serum reveal total AMH production and secretion by the full Sertoli cell population. The increase of total production of AMH after treatment of FSH could result from an increase of Sertoli cell number or of AMH gene expression, or both. While FSH effect on Sertoli cells has proven proliferative, principally in the prepubertal testis (40) (41), a rise in the Sertoli cells number secreting AMH is indeed responsible, at least in part, for the enhance in AMH levels. The present study shows high level of LH in patient. In answer to FSH, the Sertoli cells produce diverse factors that affect Leydig cell function equally, Leydig cells produced testosterone is necessary for spermatogenesis (42) (43). High concentrations of circulating LH may be suitable to hence degeneration of Sertoli cells and germinal cells, Therefore, maintaining LH serum levels is very essential for initiating and supporting spermatogenesis. The result present study is in agreement with the study that reported significantly increased LH were, while Serum levels of AMH was insignificantly reduced in infertile men comparison with those of fertile (37) . Another study concludes that there is significant reduction in serum testosterone & AMH of infertile men as compare with fertile men. However, there is significant increase in serum LH in infertile men (44).

The current study shows a significant negative correlation between AMH and LH in patients. That means the decline of AMH level is accompanied with the elevation of LH level. Since the LH receptors are founds on the Leydig cells of the testes (17) (45). Other study show that AMH has been recommended for postnatal differentiation of Leydig cells

Al-Kufa University Journal for Biology
Print ISSN: 2073-8854 & Online ISSN: 2311-6544

as a negative regulator, AMH exerts a differentiation and function of the Leydig cell by inhibitory effect due to a decreased number of mature Leydig cells, the conduct show that the AMHR expresses Leydig cells and that over expression of AMH in transgenic mice of male, blocks the precursors of Leydig cell, differentiation and the expression of steroidogenic enzyme mRNAs (37). The present study concluded that AMH is one of the key factors conditioning the normal development of male genitals. Serum AMH determination is clinically valuable in assessing gonadal function.

References

1. **Evers,** JL. (2002). Female subfertility. Lancet 360, 151–159.
2. **World Health Organization**. (1975). The epidemiology of infertility. Report of a WHO Scientific Group. Geneva.. (WHO Technical Report Series, No. 582).
3. **World Health Organization**. (1991). Infertility. A tabulation of available data on prevalence of primary and secondary infertility. Programme on Maternal and Child Health and Family Planning. Division of Family Health. Geneva: WHO; 1991.Oct.24.
4. **Safarinejad, MR**. (2008). Infertility among couples in a population-based study in Iran: prevalence and associated risk factors. Int J Androl. Jun; 31 (3): 303-14.
5. **Massague, J** .(1998). TGF-beta signal transduction. Annu Rev Biochem 67,753–791.
6. **- Massague, J.** (1990). Th e transforming growth factor-beta family. Annu Rev Cell Biol; 6: 597-641.
7. **Hampel, R. ;** Snajderova, M. and Mardesic, T. (2010). Anti müllerian Hormone (AMH) Not Only aMarker for Prediction of Ovarian Reserve (mini review). Endocrinology regul.; 30:1-7.
8. **Razich, DF**.; Naeimeh ,T. and Maryam, A. (2008). Serum level of Anti-Mullerian hormone in early follicular phase as a predictor of ovarian reserve and pregnancy outcome in assisted reproductive technology cycles. Arch of Iranian Med; 11(4): 371-376.
9. **Rodina, AV**.; Gukasova, NV.; Makarov, VA.; Kondrasheva, IG.; Khomgakov, GA.;Posypanova; Popova, ON.; Yumoskaleva,YU. and Severa, SE.(2008). Localization of Mullerian inhibiting substance receptors in various human cancer cell lines .Biochemistry (Moscow) . 75,(7) : 797-805.
10. **Behringer, R.R**.; Finegold, MJ. and Cate, RL. (1994). Mullerian-inhibiting substance function during mammalian sexual development. Cell 79,415–425.
11. **Josso, N**.; di Clemente, N. and Gouedard, L. (2001). Anti-Mu¨llerian hormone and its receptors. Mol Cell Endocrinol;179:25–32.
12. **Gruijters, MJ**.; Visser, JA.; Durlinger, AL. and Themmen, AP.(2003) .Anti-Mullerian hormone and its role in ovarian function. Mol Cell Endocrinol 211,85–90.
13. **Pierce,J.G.and** Parsons,T.F. (1981). Glycoprotein hormones: structure and funct-ion. Annu. Rev. Biochem. 50,465–495.
14. **Fiddes,J.C.;**and Talmadge, K.(1984). Structure, expression, and evolution of the genes for the human glycoprotein hormones. Recent Prog.Horm. Res. 40,43–78.
15. **Parsons, TF**.; Strickland, TW. and Pierce, JG. (1985). Disassembly and assembly of glycoprotein hormones. *Methods Enzymol*.;109:736- 749.
16. **Dias, J. A.** (2002). Molecular, structural, and cellular biology of follitropin and follitropin receptor. Vitam. Horm. 64, 249–322.

17. **Ascoli, M.; Fanelli, F.and Segaloff, D. (** 2002). The lutropin/choriogonadotropin receptor, a 2002 perspective. Endocrine reviews 23:141-174.
18. **Szkudlinski**, M.W.; Fremont,V.; Ronin, C. ;and Weintraub, B. D.(2002). Thyroid-stimulating hormone and thyroid-stimulating hormone receptor structure function relationships. Physiol. Rev. 82, 473–502.
19. **Simoni, M.;** Gromoll, J. and Nieschlag, E. (1997). The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and patho physiology. Endocr Rev.;18(6):739-773.
20. **Timossi, C.;** Maldonado, D. and Vizcaino, A.(2002). Structural determi nants in the second intracellular loop of the human folliclestimulating hormone receptor are involved in G(s) protein activation. Mol Cell Endocrin.189(1- 2):157-168.
21. **Themmen,** A. P. and Verhoef-Post, M. (2002). Semin. Reprod. Med.20,199–204
22. **Themmen**, A. P. N. and Huhtaniemi, I. T. (2000). Mutations of gonadotropins and gonadotropin receptors:elucidating the physiology and pathophysiology of pituitary-gonadal function. Endocr. Rev. 21, 551–583.
23. **Weinbauer** , **G.F** ., Craig Marc Luetjens , Manuel Simoni and Eberhard Nieschlag . (2010) . physiology of Testicular Function in : Nieschlag E, Behre HM ,Nischlag S (EDs). Analogy : Male reproductive Healthand Dysfunction .3rd ed .Berlin Heidelberg Springer- Verlag pp-11-59.
24. **Rosse**,**D.A** .; and Smith, S.M. L. (2003) .Self_association and raft localization of functional luteinizing hormone receptor .Biol.reprod . 69,1765-1770.
25. **Babu, S. R**. ; Sadhnani, M.D. ; Swarna, M.; Padmavathi, P.; and Reddy, P.P.(2004). Evaluation of FSH,LH and Testosterone levels in different sub groups of infertile males. Indian Journal of Clinical Biochemistry, 19 (1) 45-49.
26. **Zabul, J.;** Mierzejewski, W. and Rogoza, A. (1994). Usefulness of examining gonadotropin hormones and testosterone in men with abnormal semen. Ginekol-pol., 65(2): 71-74.
27. **Bergmann,** M.; Behre, HM.and Nieschlag, E .(1994). Serum FSH and testicular morphology in male infertility. Clin Endocrinol 40:133–136.
28. **Rey, R**.; Lordereau-Richard, I.; Carel, JC.; Barbet, .;, Cate, RL.; Roger, M.;Chaussain, JL.and Josso, N. (1993). Anti-mu¨llerian hormone and testosterone serum levels are inversely during normal and precocious pubertal development.J Clin Endocrinol Metab;77:1220–1226.
29. **Young, J.; Rey, R. and** Couzinet, B. (1999). Antimüllerian Hormone in Patients with Hypogonadotropic Hypogonadism. J Clin Endocrinol Metab 84: 2696-9.
30. **- Al-Qahtani, A**.; Muttukrishna, S.; Appasamy, M.; Johns, J.and Cranfield M. (2005). Development of a sensitive enzyme immunoassay for antiMüllerian hormone and the evaluation of potential clinical applications in males and females. Clin Endocrinol (Oxf); 63: 267–73.
31. **van Rooij, IA**.; Broekmans, FJ.; te Velde, ER; Fauser, BC. and Bancsi, LF.(2002) Serum anti-Müllerian hormone levels: a novel measure of ovarian reserve. Hum Reprod 17: 3065–71.
32. **Young, J.;** Chanson, **P**. and Salenave, Sl.(2005). Testicular anti-Müllerian hormone secretion is stimulated by recombinant human FSH in patients with congenital hypogonadotropic hypogonadism. Journal of Clinical Endocrinology and Metabolism, vol. 90, no. 2, pp. 724–728.
33. **Griswold, M.D.** (1993). Actions of FSH on mammalian Sertoli cells.In The Sertoli Cell. L.D. Russell and M.D. Griswold, editors. Cache River Press, Clearwater, FL. 493–508.

34. **Al-Attar,L.;** Noë;K.; Dutertre,M.; Belville,C.; Maguelone, G.; Forest; Paul, S.; Burgoyne; Josso,N.; and Rey,R. (1997). Hormonal and Cellular Regulation of Sertoli Cell Anti-Müllerian Hormone Production in the Postnatal Mouse. J. Clin. Invest. . 100:1335–1343.
35. **Meeker, JD**.; Godfrey-Bailey, L. and Hauser, R.(2007). Relationships between serum hormone levels and semen quality among men from an infertility clinic. J. Androl., 28: 397-406.
36. **Saleh,** B. O.; ALAndni, N.K.; and Khraibet,W.H. (2014). Serum total testosterone and inhibin B are the better makers of spermatogenesis than anti-mullerian hormone in oligospermic men.American Journal Rsearch Communication, 2(1): 118-124.
37. **Lukas-Croisier, C**.; Lasala, C.; Nicaud, J.; Bedecarra,´s P.; Kumar, TR.;Dutertre, M.; Matzuk, MM.; Picard, JY.; Josso, N. and Rey, R.(2003). Folliclestimulating hormone increases testicular anti-Mullerian hormone (AMH) production through Ser-toli cell proliferation and a nonclassical cyclic adenosine 5-monophosphate-mediated activation of the AMH gene. Mol Endocrinol 17(4):550–561.
38. **Kuroda,** T.; Lee, M.M. ; Haqq, C.M. ; Powell, D.M. ; Manganaro, T.F.;and Dona-nahoe, P.K. (1990). Müllerian inhibiting substance ontogeny and itsmodulation by follicle-stimulating hormone in the rat testes.Endocrinology.127:1825–1832.
39. **Voutilainen, R., and W.L. Miller**. (1987). Human Müllerian inhibitory fac-tor messenger ribonucleic acid is hormonally regulated in the fetal testis and in adult granulosa cells. Mol. Endocrinol.1:604–608.
40. **Orth, JM.** (1986). FSH-induced Sertoli cell proliferation in the developing rat is modified by b-endorphin produced in the testis. Endocrinology.119: 1876–1878.
41. **Almirón, I.;** and Chemes H. (1988). Spermatogenic onset. II. FSH modulates mitotic activity of germ and Sertoli cells in immature rats. Int. J. Andro 11:235–246 (Oxf) 63:267–273.
42. **Saez, J. M.,;** Avallet, O.; Naville, D.; Perrard-Sapori, M. H. and Chatelain, P. G (1989)Ann. N.Y. Acad. Sci.564,210–231.
43. **Saez, J. M.** (1994).Endocr. Rev.15,574–626.
44. **Wisam S**. Najam. (2012). Significant value of hormonal assays as a marker for Male Infertility in Tikrit city. Tikrit Medical Journal;18(2):314-321.

Menon, K.; Munshi, U.; Clouser, C.and Nair, A. **(**2004). Regulation of Luteinizing hormone /human chorionic gonadotropin receptor expression: a perspective. Biology of reproduction 70:861-866.