Original Article

The impact of proteins, glycoproteins and fructose in blood and seminal plasma on sperms concentration In infertile men

Nahla M. Al-Sakkal* **MSc**
Nassir M. Al-Rubae* **PhD**
Zina A. Marrow* **MSc**
Basil Y. Safah** **MSc**

Fac Med Baghdad 2009; Vol. 51, No.1
Received May 2008
Accepted Oct. 2008

Summary:

Background: proteins, glycoproteins and fructose as parameters used to assess infertility in men.
Objective: To determine and correlate serum and seminal plasma of total proteins, glycoproteins and fructose with infertility in men.
Patients and Methods: The study was performed on 154 subjects; 109 infertile men (oligospermic and azoospermic) and 45 normal volunteers (normospermic men). All sera and seminal plasma were submitted for total proteins, glycoproteins and fructose levels measurment.
Results: No significant difference was noted in serum and seminal plasma of total proteins in oligospermic and azoospermic and that of normospermic men ($P>0.05$) compared to normospermic men.
Statistical significant reduction ($P<0.05$) was noted in seminal plasma glycoproteins in oligospermic as compared to normospermic and azoospermic men.
A significant elevation ($P<0.05$) of fructose levels were observed in seminal plasma of azoospermic when compared to others.
Conclusion: This study may indicate that the higher concentration of glycoprotein in seminal plasma the better quality of semen and a significant negative correlation ($r=-0.749$: $P<0.05$) were observed between seminal plasma fructose and sperm count of infertile men.
Key words: Infertility, proteins, glycoproteins, fructose.

Introduction:

The exact role of protein in seminal plasma has not been defined, but there is some evidences that the amino acids and proteins play a role in sperm survival (1). The beneficial action of these amino acids is believed to be their metal-binding capacity. Free amino acid represent an extracellular oxidizable substrate for the aerobic metabolism of spermatozoa, this possibility arises mainly from the discovery of amino acid oxidase in spermatozoa (2). Free amino acids are utilized by sperm cell for protein biosynthesis. Spermatozoa contains a minute but significant amount of true ribonucleic acid which is of a special type and resembles messenger (RNA) these being able to mediate the process of spermatozoal protein synthesis(3). Herrmann (1998) (4) has demonstrated the precence of 11 types of serum proteins in human seminal plasma. The selenoproprotien phospholipid hydroperoxide glutathione peroxidase ($PHGP_X$) accounts for almost the entire selenium content of mammalian testis. Male infertility in selenium protein deficient animals is considered to result from insufficient $PHGP_X$ content (5).

** From the department of Medical Biochemistry, College of Medicine, Al- Mustansiriya University*
*** From the department of Biochemistry, College of Medicine University of Mosul*

The seminal sugar was isolated for the first time from ball semen and identified as for D (-) fructose. Montagnon et.al, (6) believes that seminal fructose forms light bounds with certain proteins that are of vesicular origin. The main site of fructose formation is the seminal vesicle yet; an additional small amount comes from the ampullary glands. Fructose synthesis is hormone –dependent process, it disappeared almost completely within 2 weeks after castration, while was prevented or restored by implantation of testoterone, since seminal vesicle are testosterone dependent (7).The seminal fructose concentration is primarily an indicater of the size, storage and secretary capacity of the seminal vesicles and of human androgenic activity (8). Biosynthesis of seminal sugar involves the conversion of blood glucose into seminal fructose (9). Apart from the phosphorylation pathway of glucose metabolism, the male accessery organ of ram and rat possess an altenative, non phosphorylation mechanism for the conversion of glucose to fructose which involves sorbitol as an intermediary metabolites and sorbitol dehydrogenase (ketose – reductase) as an intermediary enzyme(10).
The main function of fructose in semen is to supply the life energy to the spermatozoa in the form of an early glycolyzable material. The incubation of a freshly ejeculated whole semen is followed by a progressive fall in fructose.In species where fructose

is a normal constituent of seminal plasma, the anaerobic fructolysis is the metabolic process which enable the spermatozoa to survive without oxygen, since mammalian spermatozoa posses only negligible reserve of intracellular glycogen, they must depend under anaerobic conditions on the extracellular source of carbohydrate in the form of seminal fructose (11). The aim of our study is to evaluate the role of protein, glycoprotein and fructose in serum and seminal plasma of infertile patients.

Methods:

A sample of 154 males patients aged 20-47 years were included in this study. The patients were chosen from 200 males patients during their attendance the infertility clinic in Al- Elwiya Maternity Teaching Hospital in Baghdad after being unable to achieve pregnancy for at least a year, and for whom their partners have shown no diagnosed cause of infertility. All the selected patients were found free from any infections or endocrine disordes according to clinical examination and necessary blood analysis. The ejaculate (by masturbation) and venous blood were sampled for each subject, all of whom were required to avoid any sexual activity in the preceding 4 days. The seminal fluid was separated from the spermatozoo by centrifugation at 3000 r.p.m for 10 min at room temperature. An aliquot of the seminal fluid was decanted and stored at – 20 C° until required for analysis. Sperms concentration analyses were performed according to the procedures recommended by WHO (1992) (12).

According to the number of sperm, the patients were classified into three groups, namely:

1. Normozoospermic ($n>20x10^6$ sperm/ml) number of patients = 43
2. Oligozoospermic ($10 < n<40$ $x10^6$ sperm /ml) number of patients = 66
3. Azoospermic (n = zero) number of patients = 45

Total proteins were determined both in seminal plasma and serum according to the Biuret method using Diamond kit – Jordan (13).

The glycoprotein was determined according to hexose moiety of protein – carbohydrate conjugate precipitated by 95% ethanol at room temperature, and determined by the orcinal reaction both in seminal plasma and serum (14).

Fructose was estimated in seminal plasma according to the method described by Mann (11).

All measurements of blood and seminal fluid were performed in duplicate.

Statistical analysis was performed, the results were expressed as mean ± SD. Comparison between data was made using the student's t-test.

Results:

Table (1) and figure (1) show the Mean, ± SD and ranges expressed in g/dL of seminal plasma and serum total protein (TP) of infertile patients compared to fertile men. There was no significant differences (P>0.05) in seminal plasma and serum TP levels of oligospermic (4.8 ±0.92 and 6.9 ±0.73) or azoospermic (5.1 ± 0.81 and 7.0 ±0.77) patients and that of normospermic men 5.0 ±0.88 and 6.8 ±0.71). The study reveals a significant positive correlation between serum and seminal plasma TP of infertile patients (r =+ 0.771, P<0.01).

Table (1): Total protein levels in seminal plasma and sera of infertile patients compared to normospermic men (Mean ±SD).

Total protein g/100 ml	Normospermia	Oligospermia	Azoospermia
	Mean ±SD (Range)	Mean ±SD (Range)	Mean ±SD (Range)
Sample size	43	66	45
Seminal plasma level	5.0±0.88 (3-6.2)	4.8±0.92$^+$ (2-6.1)	5.1±0.81$^+$ (3.2-6.6)
Serum level	6.8±0.71 (6.3-8.3)	6.9±0.73$^+$ (6.0-9)	7.0±0.77$^+$ (6-8.8)

+: No significant difference with normospermic men.

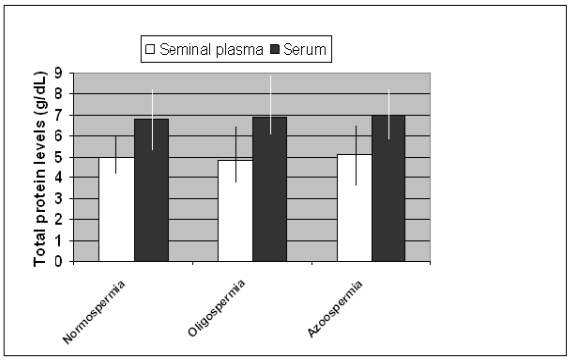

Figure (1): Seminal plasma and serum total protein levels of oligospermic and azoospermic patients compared to normospermic men (Mean±SD)

Seminal plasma and serum glycoprotein, mean values ± SD and ranges expressed as mg/dL are shown in Table (2) and figure (2), statistically significant (P<0.05) reduction in the level of seminal plasma glycoprotein has been shown in oligospermic patients (110 ± 13.5) as compared to both normospermic men (145.6 ± 8.7) and azoospermic patients (141.9 ± 22.3). The results of this study also demonstrated that the seminal plasma glycoprotein levels shows a positive relationship (r=+0.748, P< 0.01) to the sperm count. Table (3) and figure (3) show the mean levels, ± SD and range expressed as mg/dL and statistical significance of seminal plasma fructose levels of infertile patients both with azoospermic (429.6 ± 84.7), oligospermic (330.3±46.8) patients and fertile men (287.8 ± 47.5). The study shows a statistical significant elevation (P<0.05) in seminal plasma fructose levels of azoospermic and oligospermic patients compared to that of nomospermic men. Also a significant elevation (P<0.05) of fructose levels were observed in seminal plasma of azoospermic patients (P<0.05) when compared to oligospermic patients and a significant negative correlation of seminal plasma

fructose levels to the sperm count (r= - 0.749, P<0.01).

Table (2): Glycoprotein levels in seminal plasma and sera of infertile patients compared to Normospermic men (Mean ±SD).

Glycoprotein mg/100 ml	Normospermia	Oligospermia	Azoospermia
	Mean ±SD (Range)	Mean ±SD (Range)	Mean ±SD (Range)
Sample size	42	66	45
Seminal plasma level	145.6±8.7 (125-167)	110.0±13.5** (90-145)	141.9±22.3+ (90-200)
Serum level	120±21 (85-165)	115.5±18.6+ (78-160)	118.9±21.4+ (85-160)

* P value < 0.05 in comparison to normospermic men.
** P value < 0.05 in comparison to azoospermia patients.
+: No significant difference with normospermic men.

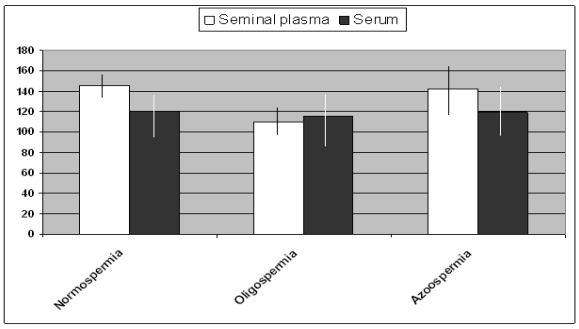

Figure (2): Seminal plasma and serum Glycoprotein levels of oligispermic and azoospermic patients compared to normospermic men (Mean ±SD).

Discussion:

Impaired fertility of the male partner is causative or contributory to in up to one half of all couples unable to conceive spontaneously. Seminal fluid fulfils a dual role; it provides optimal conditions for fertilization and protects male germ cells from infections (15). Protein content in serum and seminal plasma has been studied (16, 17). Workers concluded that the low concentration of total protein in seminal plasma of infertile male was due to absence of vesiculer proteins with higher iso-electric pH above 8.3, and that in azoospermic the number of protein bounds decreased both toward cathode & anode. Protein phosphorylation is involved in sperm capacitation. It was suggested that in vitro treatment of spermatozoa with inhibitors of protein phosphatases may be of great value in some cases of unexplained infertility (18). Granz et al., 1993 (19) found a significant increase in seminal plasma T. protein level of infertile men compared to controls. Our results in Table (1), Fig (1) show no significant differences (P>0.05) in seminal plasma and serum of the three groups.These finding may be due to wide range of protein level or the elevation in the level of some fractions of T. protein (e.g Immunoglobulins) . Stauffer and Parsons (1989) (20) studied proteins evidence, found that glycoproteins responsible for cell–to-cell interaction, could be a subfertility factors and contributed to male infertility, and their results show the mean glycoprotein level is significantly higher (P<0.05) for vasoctomy group. Furthermore, disc electrophoresis of prostatic secretion glycoproteins in polyacrylamide gel has been employed by Mikhalichenkov et al., (1998) (21). Their study revealed that the progress of the inflammatory process in the prostate is associated with a drastic decrease in concentrations of prostatic secretion glycoproteins with a higher molecular weight and an increased level of low molecular glycoproteins and that these changes have not been seen in both normal fertile and infertile subjects without prostatitis. Elliot and Cooke (1997) (22), reported that spermatogensis is an elaborate process involving cell division, differentiation, and cell–cell interactions. Defects in any of these processes can result in infertility, and in some cases these can be genetic in cause (23). Semen contain fructose presumbly as a source of energy for spermatozoa and it has been reported by (WHO) as the most important biochemical marker for seminal vesicle disease (24). Comhaire and De–Bacquer, 2002 (7) indicated that true corrected seminal fructose level is a marker of the function of seminal vesicles in infertile men. Seminal fructose showed to be completely absent in infertle men with congenital absences of both vase deferentia. Tomasewski et.al, 1992 (25) reported that the difference in frequency of fructose appearance in semen of fertile and infertile men suggests that fructose may be in some ways involved in the process of fertilization(25). The present study (Table (3), fig (3)) confirm and agree with previous reports considering the levels of seminal plasma fructose. It is obvious that they increase with decrease of sperm count in the two groups of infertile men and fertile men (26, 27). This could explain on the bases of utilization of fructose by spermatozoa. The present results could explain on the possibility of feedback mechanism of low spermatogenesis which causes an increase in pitutary gonadotrophins which stimulate testosterone production, which in turn induces fructose synthesis (28). Half of the men in this study were smokers. Cigarette smoking was associated with reduced semen quality, but fructose concentrations were slightly but none significantly affected (29).

Table (3): Fructose levels in seminal plasma of infertile patients compared to Normospermic men (Mean ±SD).

Fructose mg/100 ml	Normospermia	Oligospermic patients	Azoospermia patients
	Mean ±SD (Range)	Mean ±SD (Range)	Mean ±SD (Range)
Sample size	43	66	45
Seminal plasma level	287.8±47.5 (127-362)	330.3±46.8* (210-410)	429.6±84.7** (280-560)

* P value < 0.05 in comparison to normospermic men.

** P value < 0.05 in comparison to Oligospermic patients.

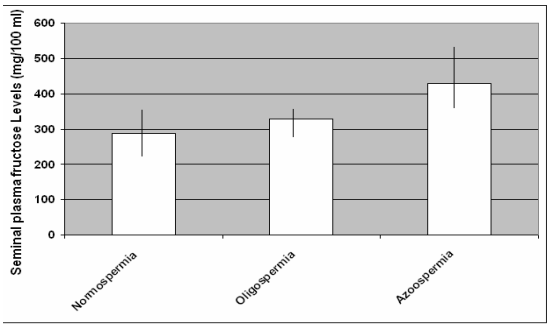

Figure (3): Seminal plasma fructose levels of oligospermic and azoospermic patients compared to normospermic men (Mean±SD)

References:

1. Tyler, A. and Rothschild: Metabolsim of spermatozoa and induced anaerobic motility in solution of amino acids. Proc. Soc. Exp. Biol. N. Y.1998, 76:52.

2. Giarola, A. and Ballerio, C.: Biochemistry of serum proc. III world congress on fertility and sterility international congres series No. 35, Excerpta foundatio, Amesterdam, 1999, 281.

3. Ghosh, I; Bhardwaj A. Reduction in the level of hyaluronan binding protein Iis associated with loss of sperm motility. J. Reprod. Immunol , 2002, 53 (1-2):45-54.

4. Herrmann, W.P. and Hermann, G.: chromatograpische franktionierung and Immunologische analyse Von menschlichen. Spermaplasma. Arch Klin Exp. Dermatal, 1998, 234:100.

5. Foresta C, Flohe L, Garolla A et.al, Male fertility is linked to the selenoprotein phospholipid hydroperoxide glutathione peroxidase. Biol. Reprod, 2002, 67(3): 976-71.

6. Montagnon, D, I Clavert, A. and Cranz,C.Fructose, proteins and coagulation in human seminal plasma. Andrologia, 1983, 14:434.

7. Comhaire F., De-Bacquer C. True corrected seminal fructose level: a marker of the function of seminal vesicles in infertile men. Int. J. Androl, 2002; 25(4): 253.

8. Vonder Kammer, H; Scheit, K.H.; Weidner, W. and Cooper, T.G.: the evaluation of markers of prostatic function. Urological Research, 1991, 19:343-7.

9. Kuhlman, K.E.: Hexose – 6 – phospatase activity in seminal vesicles and prostate of rat. Proc. Soc. Exp. Biol. Med. N. Y, 1990, 85:458.

10. Patel, V.: Degradation of glycoprotein. Horowiz, M. and Pigman. W. (eds) the glycoconjucates. Vol. 12, Academic press, 1995, 12,387.

11. Mann, T.: Fructose and fructolysis in serum in relation to fertility. Lancet, 1998, 24:446.

12. World Health Organization.Manual for the examination of human semen. 1992 Cambridge University press, 3rd edn.

13. Silverman LM et al., Amino acid and proteins. Tietz N.W 2nd Textbook of clinical chemistry U.B. Sauders 1986, ch 4 p519.

14. Varley, H, Gowemlock, A.H. and Bell, M: practical clinical biochemistery 5th ed. William Hermammann Medical Book, LTD, 1964 vol.1 ch.25 P.850.

15. Hampl R et al., The content of four immunomodulatory steroids and major androgen in human semen. J. steroid Biochem. Mol. Boil 2003; 84(2-3), 307-16.

16. Autiero, M., Sansone, G, Abesuia, and P.: Relative ratio of lactoferin; albumin, and acid phosphatase sminal level as sperm quality marker in fertile and infertile men. Indian. J. Med. Sci, 1991, 47(3):61-67.

17. Verma, P.K., Singh, J.N., Guadrose, M. (1993): Modifications in the seminal protein pattern and concentration among infertile men. Indian J. Med. Sci, 1993, 47(2):50-54.

18. Hortas ML et al., Alterations in sperm protein phosphorylation in male infertility Andrologia 2001, 33(5): 282-6.

19. Cranz, C., Belmekki, M., Clavert, a: Inflammatory protein of seminal fluid. Contracept fetil sex, 1993, 21(5): 378-379.

20. Stauffer, C.W. Parasons. C.L. Fibronectin levels in male ejaculate and evidence for its role in unexplained infertility. Urology 1989; 34(4); 80-85.

21. Mikhalichenko, V.,; Pupkova L., Kozlova A.: Electrophoretic study of protein secretion by the prostatic gland in chronic prostitites and pathospermic. Lab. Delo, 1998, 4:8-11.

22. Eliott, D.J and Cooke, H.J. The molecular genetic of male infertility. Bioassays, 1997, 19 (9):801-809.

23. Dohle GR. Halley DJ, Van-Hemel J et al., Genetic risk factors in infertile men with severe oligozoospermia and azoospermia Hum. Reprod. 2002; 17(1): 13-6.

24. Lewis-Jones, D.I., Aird, I.A., Bilijan, M.M; Kingsland, C.R.: Effect of sperm activity on zinc and fructose concentrations in seminal plasma. Human Reprod, 1996, 11:24-65.

25.TomasZewski, L.; Konarska, L.; Janczewski, Z.; sharzynska, E.; Lbioda, K.; Hryckiewiez, L.: Fructose in human and bovine semen. Life. Sei, 1992, 50(3): 181-185.

26. Rocha, F.T.: Seminal fructose levels in male infertility relationship with sperm charachteristic. Int. Urol. Nephrol, 1999, 31(1): 107-111.

27. Andrad-Rocha., F.T.: fructose in the semen and its relation with the sperm count. Reproduca, 1993, 8:16.

28. Moon K. Osborn RH, Yannone ME et al, and Relationship of testosterone on human seminal fructose. Invest. Uro. 1970, 7: 478.

29. Kunzle R. Mueller MD. Hanggi.w ET. al., Semen quality of male smokers and nonsmokers in infertile couples. Fertil- Steril 2003; 79(2): 287-91.