

# FITC – LECTIN BINDING PROFILES ON SPERM STRUCTURES OF FERTILE AND INFERTILE MEN WITH AND WITHOUT OBESITY

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**Abstract—** In this study the distribution of binding sites of Concanavaline A (Con A), Wheat germ agglutinin (WGA), Ulex europaeus agglutinin (UEA) and Peanut agglutinin (PNA) lectins conjugated with fluorescence isothiocyanate (FITC) with different specific carbohydrates were studied on cell surface of sperms of fertile and infertile men to determine whether the surface characters of the spermatozoa of fertile men differs from that of infertile men with or without obesity.

A total of 255 infertile and 319 fertile men were involved in this study between Sep. 2006 and Dec. 2008. The body mass index (BMI) was measured in all fertile and infertile men. All semen parameters were assessed in both fertile and infertile men with or without obesity to identify the values that would distinguish fertile from infertile men.

The results of this study showed that there is a clear significant difference between fertile and infertile men with or without obesity in regard to all semen parameters. On the other hand, all lectins used in this study were reacted differentially with the various components of the sperms of fertile and infertile men with or without obesity. This suggests that the obesity might alter the cell surface changes of sperms with increasing of BMI.

**Key words—** LECTIN, Concanavaline, fluorescence isothiocyanate, semen parameters etc

## I. INTRODUCTION

Infertility is a condition that affects both men and women of the reproductive age. Infertility is the diminished ability or the inability to conceive and have offspring after one year of unprotected coitus or sex relation. Infertility may be designed as either primary or secondary. Primary infertility is the term used to describe a couple a couple that has never been able to conceive a pregnancy after at least one year of attempting to do so through unprotected intercourse. Secondary infertility is the term used when a couple has conceived previously, but is unable to conceive again (Check, 1995).

Infertility can be due to many causes, as many studies have shown that more than half of causes of infertility are a result of female conditions; the remainders are caused by sperm disorders and by unexplained factors. Male infertility could be caused by many factors such as hormone disorders, illness, reproductive anatomy trauma and obstruction, and/or sexual dysfunction. These factors can temporarily or permanently affect sperm and prevent conception (Harvey, 2008).

Infertility nowadays is on the rise between obese couples in many countries. Obesity immediately brings to mind association with hypertension, diabetes and heart diseases (Manson et al., 1990). Yet most people are surprised to learn that there is an association between obesity and infertility. Epidemiological data confirm that obesity accounts for 6% of primary infertility. Thus 12% of primary infertility result from deviations in body weight from established

norms (WHO, 1987). On the other hand, the presence of carbohydrates containing materials on cell surfaces has long been known (Topfer – Petersen et al., 1990). Many investigators have demonstrated that cell surface carbohydrates and complementary lectins (sugar binding macromolecules) on opposing cell surface mediate cell-cell adhesion in vitro (Barros et al., 1988). The current interest in lectins is not just because of their usefulness in detecting and studying carbohydrate on cell surface, but also because lectins are believed to serve as recognition determinants in a variety of systems including the binding of sperm to zona pellucida of the egg (Forsman and Silva et al., 1989). Sperm egg binding is commonly highly specific and depends on cell – surface molecules on the sperm and egg for its fidelity. In recent years, a comprehensive range of lectins labeled with FITC have been used as histochemical reagents to study anatomically normal tissues (Nicolson et al., 1977). For example, considerable information has been accumulated about the binding of lectins to the cell surfaces of sperm and egg (Centrola et al., 1990). But they did not mention whether there is a difference in lectin binding to cell surface of the sperms or eggs in fertile and infertile couples with obesity. Therefore this study was undertaken in an attempt to find out whether or not the obesity may alter the cell surface composition of the sperm membrane using an FITC - lectins.

## II. MATERIALS AND METHODS

### A. Population

Total of (255) infertile patients were included in this study between Sep 2006 and Dec 2008. 203 out of this number were obese. The fertile patients were distributed as 145 for primary infertility and 110 for secondary infertility. Primary infertility men with obesity were 113, whereas the secondary infertility men with obesity were 90. Only the primary infertility men were involved in this study. The average age of primary infertile men was 36 years. This study was also included 319 fertile men either with obesity (265) or without obesity (54). The average age of fertile men was (31) years, there sperms were considered as a positive control either for semen parameters or for FITC – lectin binding cell surface of sperms.

### B. Collection of data

The data of this study was collected through interviewing of the fertile and infertile men during their first and second visit in infertility clinic and IVF center in Pediatric Hospital and Rizgary Laboratory Hospital in Erbil City, Iraq.

### C. Semen Collection

The procedures for semen collection after 3-5 days of sex abstinence are based on WHO recommendations (WHO, 1987, 1992 and 1999).

### D. Semen parameters

Semen volume, sperm liquefaction, sperm motility, sperm concentration and sperm count were done within 30-60 minutes of 37 incubated semen based on normal range of WHO recommendations: volume= 2-6 ml, liquefaction= < 60 min, sperm concentration= 20 million sperms/ml or more, sperm count= 50% or more with forward progression and 25% or more with rapid progression within 60 minutes and morphology= 30% or more with normal forms (WHO, 1987, 1992 and 1999).

#### *E. Preparation of sperms for eosin – nigrosine staining*

Human semen samples were obtained from fertile and infertile men with or without obesity according to WHO 1987 after 2 – 3 days of sexual abstinence. The method of Jaiswal et al., 1999 was applied with some modification. Following incubation the supernatants containing sperms were divided into three aliquots (at least 50 ul for each). One for staining with eosin – nigrosine, one used as a control using both lectin and its specific carbohydrate altogether. The other only with FITC – lectins.

#### *F. Measuring of BMI*

In this study, the amount of body fat was assessed clinically using the BMI formula by Cole et al., 1995).

#### *G. Staining procedures of sperms with FITC – lectins:*

Following preparation of sperms, 10 ul of sperm aliquot in a test tube was incubated with 10 ul of 500ug/ml of each of the following lectins: Concavaline A (Con A), specific to mannose; Wheat germ agglutinin, specific to N- acetyl- glucosamine; Ulex europaeus agglutinin, specific to &-L-fucose and Peanut agglutinin, specific to galactose (Goldstein and Hayes, 1978) for 30 minutes at 22 C in dark chamber. Following incubation the mixture was centrifuged (300 g for 10 minutes) and the suspension containing sperms was washed twice through PBS in the same manner to remove the unbound lectins. Thereafter an aliquot (10 ul) of spermatozoa was smeared onto the surface of the glass and allowed to air dry. Finally the slides were mounted with hydromount and a cover slide prior to examination by epifluorescence microscopy. For control, in addition to FITC – labeled lectins, equal volume of the corresponding monosaccharide (specific carbohydrate) was added to one of the aliquot.

Intensity of staining was scored visually according to the following of FITC brightness: +++ intense, ++ strong, + Moderate, +\_ weak and – no.

### III. RESULTS

Semen characteristics of the infertile and fertile men regarding to their BMI. In this study the distributions of the BMI in 574 infertile and fertile men were as following: 103 (17.9%) as a normal weight, 291 (50.7%) over weight and 180 obese. The number of fertile men with and without obesity was 319. These samples were divided into five groups according to their BMI with their number: 18.5 – 24.9 (54), 25 – 29.9 (187), 30 – 34.9 (59), 35 – 39.9 (12) and > 40 (7), respectively. While the number of infertile men with or without obesity was 255. These samples also were divided into five groups according to BMI with their number: 18.5 – 24.9 (61), 25 – 29.9 (99), 30 – 34.9 (44), 35 – 39.9 (20) and > 40 (31), respectively.

Regarding semen liquefaction, sperm volume and sperm count, the results of this study showed a significant difference between infertile and fertile men (P- value=0.00). There was an inverse relation between BMI groups and these parameters; they were decreased as BMI groups increased.

Concerning the sperm motility, the current results revealed a significant difference between the sperm active of fertile and infertile

men (P- value=0.00). Sperm active decrease as BMI increased. Significant differences were recorded between the sluggish sperms of fertile and infertile men (P- value=0.034). There was a positive relation between BMI and sluggish sperm percentage (P- value=0.005). No significant differences were found between BMI groups in the percentage of immotile sperm with P- value=0.365. The interactions show no significant differences between immotile sperms of infertile and fertile men.

In this study, there was a positive relation between BMI and value sperm morphology. It means that the normal sperm morphology decreases with increases of BMI (P- value =0.049). With regard to abnormal sperm morphology, a significant difference was found between infertile and fertile men as well as between different BMI with the P- value= 0.00 and 0.01, respectively.

#### *A. FITC – lectins binding profiles of sperm structures of fertile and infertile men with or without obesity*

Results of FITC – conjugated lectins binding to the sperm structures of fertile and infertile men with or without obesity are shown in Table 1.

Con A showed strong and moderate staining with all anatomical structures of the sperms of non-obese and obese fertile men, respectively (Plate1 and Plate 2). The reactivity of Con A with all structures of sperms of non-obese infertile patients was unclear due to the presence of random spots of FITC on most fields. However all structures showed weak FITC with this lectin (Plate 3). Regarding the sperms of obese infertile men, this lectin showed a very weak staining with acrosome adjacent to the head region. But no binding was found on head, neck and tail (Plate 4)

WGA revealed intense binding with all components of sperms of non – obese fertile men (Plate 5), whereas the reactivity of this lectin with the sperms of obese fertile men was moderate with the head and negligible with neck and tail (Plate6). The reactivity of this lectin with sperm's structures of none - obese infertile men was similar to that of Con A lectin binding none – obese infertile men (Plate 7). Binding of this lectin to the head, neck and tail of sperm of obese infertile patients was also negative except that there was a weak staining with the acrosome (Plate 8).

In this study, although the distribution of UEA was unclear because of random spots present on most fields, this lectin showed a strong binding with different structures of sperms of non – obese fertile men (Plate 9 ), whereas the sperms of fertile men with the obesity revealed a moderate binding with the head and negative with both the neck and tail (Plate 10). Regarding the sperms of none – obese infertile men, the reaction of this lectin was moderate but not homogenous especially with the heads. Neither the necks nor tails showed binding of this lectin (Plate11). The reactivity of this lectin with the sperms of obese infertile men was similar to that of Con A and WGA (Plate 12).

The fourth lectin PNA expressed a strong staining with all structures of sperms of non – obese fertile men (Plate13). The distribution of this lectin with sperms of obese fertile men was also unclear due to the random spots of FITC on the slides, however only the head showed moderate staining (Plate 14). Regarding none – obese infertile men, this lectin showed strong staining with most heads of their sperms but weak with neck and tails (Plate 15), whereas the binding of this lectin with all structures of the sperms of obese infertile men was negative (Plate 16).

Condition	FITC – lectins binding sperm structures											
	Con A			WGA			UEA			PNA		
	Head	neck	tail	Head	neck	tail	head	neck	tail	head	neck	tail
Fertile non obese	++	++	++	++	+++	+++	++	++	++	++	++	++
Fertile obese	++	++	++	+	-	-	+	-	-	+	-	-
Infertile non obese	+ <sub>-</sub>	+ <sub>-</sub>	+ <sub>-</sub>	+ <sub>-</sub>	+ <sub>-</sub>	+ <sub>-</sub>	***	-	-	++	+ <sub>-</sub>	+ <sub>-</sub>
Infertile obese	-*	-	-	-*	-	-	-*	-	-	-	-	-

\*Weak fluorescence was restricted on acrosome around the head region.

\*\*Moderate staining was noticed on a few heads of sperms in every filed.

A minimum of ten sperms, prepared on at least six separate occasions from different infertile or fertile patients with or without obesity, were scored for each lectin.

Intensity of staining was scored according to the scale described in staining procedures of sperms with FITC – lectins section.

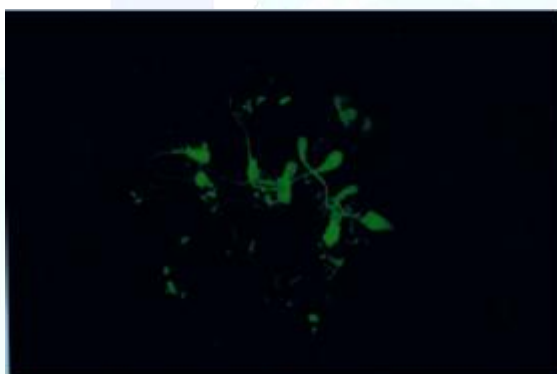


Plate 12 FITC – Con A lectin binding human sperms of non-obese fertile man All structures show strong staining. X 400

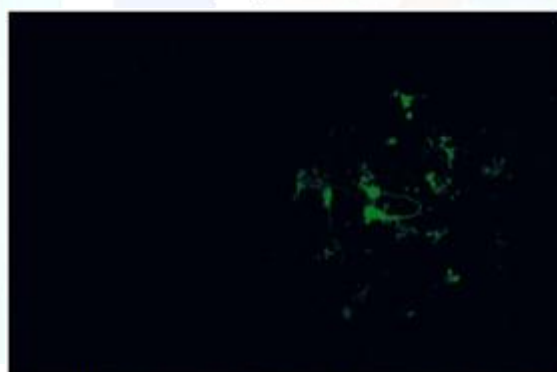


Plate 13 FITC – Con A lectin binding human sperms of obese fertile man All structures show moderate staining. X 400

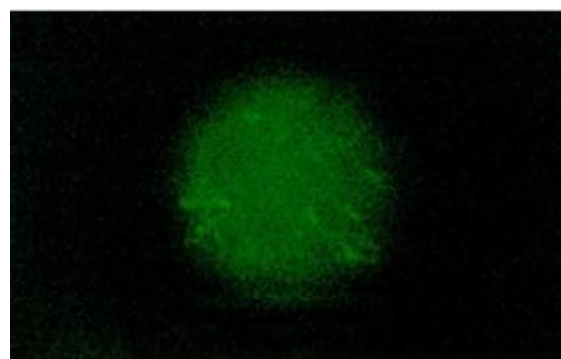


Plate 20 FITC – Con A lectin binding human sperms of non-obese infertile man. All structures show weak fluorescence. X 400

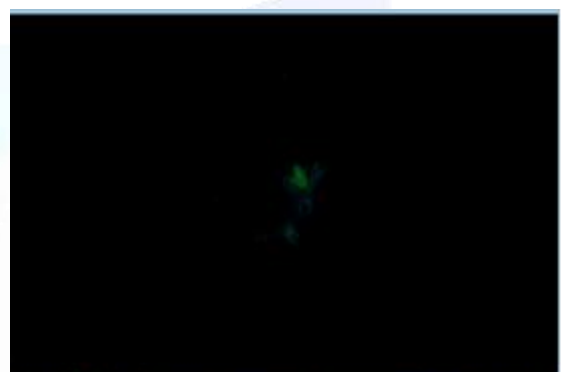


Plate 21 FITC – Con A lectin binding human sperms of fertile man with obesity. Only the acrosome shows weak staining. X 400

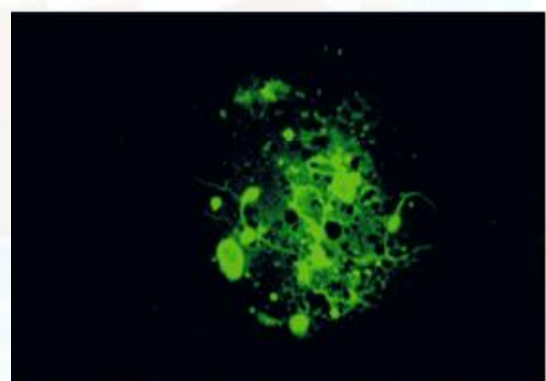


Plate 14 FITC – WGA lectin binding human sperms of non-obese fertile man. All structures show intense fluorescence. X 400

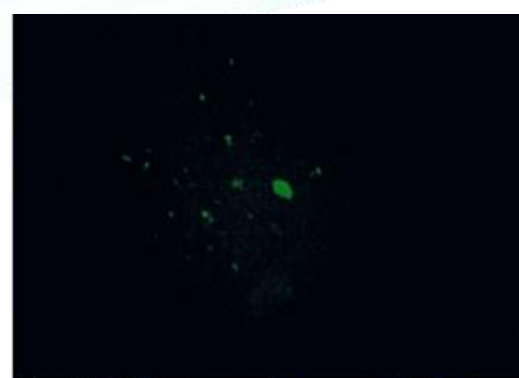


Plate 15 FITC – WGA lectin staining human sperms of obese fertile man. Only the head show moderate binding. X 400



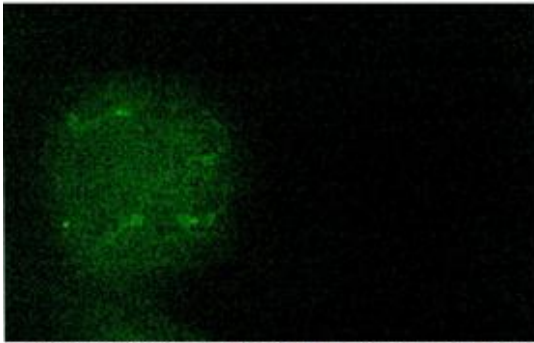


Plate 22 FITC—WGA lectin binding human sperm of non-obese infertile men. All structures show weak fluorescence. X 400

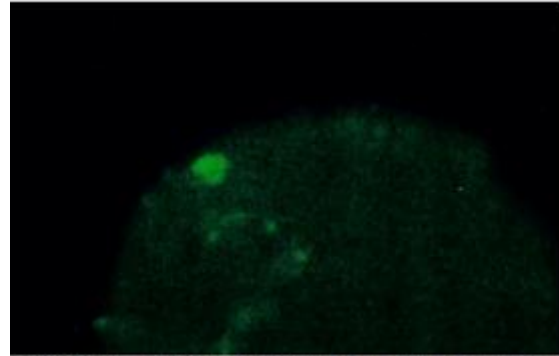


Plate 26 FITC—UEA lectin binding human sperm of non-obese infertile man. Only the head shows moderate but not homogenous staining X 400



Plate 23 FITC—WGA lectin binding human sperm of obese fertile man. Only the acrosome shows weak staining. X 1000

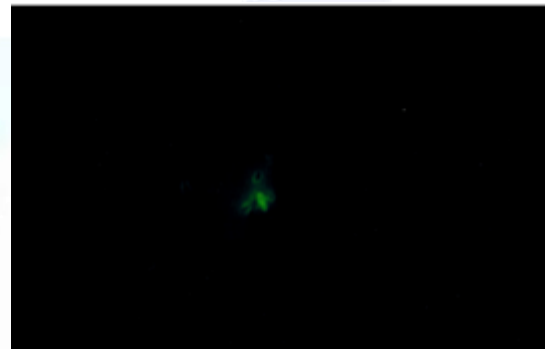


Plate 27 FITC—UEA lectin binding human sperm of infertile man with obese. Only the acrosome shows weak fluorescence. X 400

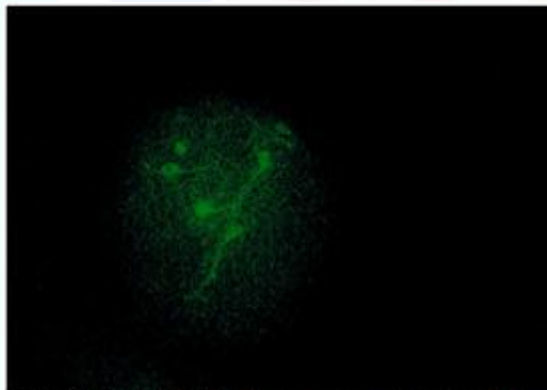


Plate 18 FITC—UEA lectin binding human sperm of non-obese fertile man. All structures show strong staining. X 400



Plate 16 FITC—PNA lectin staining human sperm of non-obese fertile man. All structures show strong fluorescence. X 1000

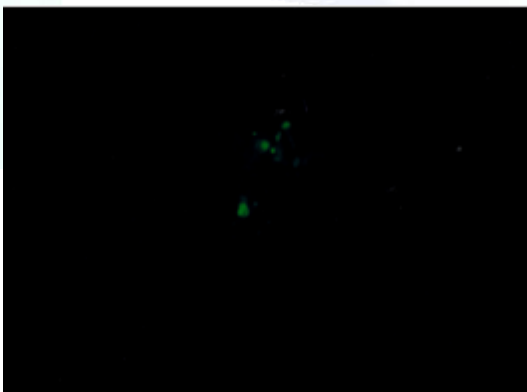


Plate 19 FITC—UEA lectin binding human sperm of obese fertile man. Only the head shows moderate staining. X 400

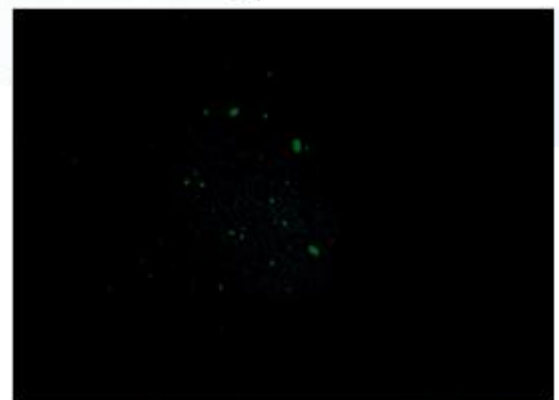


Plate 17 FITC—PNA lectin staining human sperm of fertile man with obesity. Only the head shows moderate binding. X 100

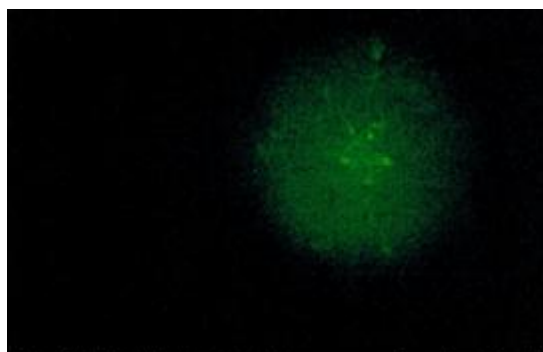


Plate 24 FITC—PNA lectin staining human sperms of non-obese infertile man. Only the head shows strong fluorescence, whereas the neck and tail show weak fluorescence.

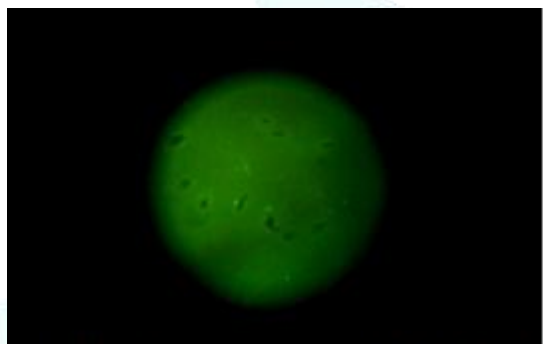


Plate 25 FITC—PNA lectin staining human sperms of infertile man with obesity. All structures show no fluorescence X 400

#### IV. DISCUSSION

In this study the semen parameters in infertile men were significantly different from that of fertile men. Sluggish sperms motility percentage and abnormal sperms morphology increased significantly in infertile patients, while the other semen parameter showed significant reduction. The influence of BMI on semen parameters was obvious except in percentage of immotile sperm. These results are in agreement with several studies (Hilton et al., 2005; Okonofua et al., 2005; Chavarro et al., 2009; Venkatesh et al., 2009). Taken together, these results suggest that evaluation of semen parameters may be related to disturbance happened due to many factors such as hormonal like FSH, LH, testosterone, estrogen, and seminal microbial infection. The imbalance in hormonal system is associated with infertility, especially the azoospermia (Kasturi et al., 2008). Other studies suggest that sperm abnormality is either through alteration of sperm membrane or directly affect DNA and genetic materials of sperm which leads to sperm damage in most cases of infertility (Kelton, 2008;). Moreover, in this study the positive relation between high BMI groups and high abnormal sperm morphology may be due to the accumulation of adipose tissue within groin region results in heating of the testicles which has been linked with oxidative stress (Banks et al., 2005).

Concerning the FITC – lectins binding sperms, the results of this study indicate that all lectins reacted differentially with the various components of the sperms of the fertile men with or without obesity. This presumably reflects the widespread occurrence of the ligands for these lectins in membrane associated glycoconjugates. Data with these lectins are consistent with the observation of Sarah et al., 2004.

With regard to FITC – lectin binding profiles of sperms structures of in fertile men with or without obesity. All lectins also reacted differentially with various components of the sperms but to a lesser extent comparing with that of fertile men. Con A specific to mannose

and WGA specific to N- acetyl- glucosamine showed moderate staining to all regions of non- obese sperms. PNA specific to galactose gave a strong binding on head and moderate on both the neck and tail. UEA specific to &-L-fucose showed only a moderate binding on the head. Moreover, the results of this study showed that when sperms of obese infertile men were treated with the four lectins, the binding of all lectins with all regions of sperms was negative. These changes in staining patterns, particularly those lectins in which a region of staining is lost in the sperm population of obese infertile men can be the result of removal of surface – adhered material in biological membranes .Brown and London, 1998) suggest that the protein redistribution to different lectins may be a part of the changes that are required for sperm to be full component to interact with oocyte and complete fertilization.

In conclusion, semen analysis alone is not a test for fertility, but carbohydrates or proteins on cell surface spermatozoa could play a role in fertilization. The shift in the staining patterns of fertile and infertile men correlated with BMI suggests that changes in distribution of cell surface proteins increasing BMI constitute part of the molecular changes which results in changes in sperm function. Furthermore lectins conjugated with FITC provide a useful tool for identifying the status of sperm population as well as to observe changes in plasma membrane components.

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