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Predictors For TESE Outcomes and Fertility Potentials Among Infertile Adult Men

Wael Zohdy, Ahmad Motawi, Mohamed omar fahmi, Adham Zaazaa

Department of andrology Faculty of medicine al kasr Al ainy Cairo university.

**Corresponding author's E-mail: mohamedomar1018@gmail.com*

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Abstract

Background: *Spermatogenesis is an essential process for human reproduction. Gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone play vital roles in the development and maturation of sperm. Growth hormone (GH) is thought to play a role in the reproductive system of both males and females. Growth Hormone deficiency can lead to reproductive problems .*
Aim: to assess predictors of fertility potentials and TESE outcomes among adult males.
Methods: *we enrolled 162 males and assessed FSH, LH, basal GH, clonidine (GH) stimulation test one time and insulin stimulation test in another time. We designed a predictive model to identify the fertility potentials, Fertility Score= 4.442 + (Basal GH*0.074) + (GH_CLON*0.035) - (FSH*0.021) (BMI*0.062)-(Smoker*0.429). The net result of this equation should be approximated to the nearest integer to predict the fertility status where, 1=TESE Negative, 2= TESE Positive, 3=Oligozoospermia, and 4= Fertile control .*
Results: *multivariate analysis showed smoking status, testicular volume, BMI, Serum FSH, basal GH are not predictors for fertility potentials. GH after clonidine and after insulin stimulation GH after clonidine stimulation correlates positively with total motile count. Other semen parameters do not correlate with basal GH or GH after insulin or clonidine stimulation. Receiver Operator Characteristic (ROC) curve analysis is used to detect the cut off levels at which sperm recovery yield change. For the GH assessment only, the basal GH could be applied to predict the SRR in men with azoospermia, AUC=0.672 (95% CI: 0.499 to 0.844). Growth hormone after clonidine (AUC= 0.510) or Insulin stimulation (AUC=0.556) and therefore, cannot differentiate between TESE positive and TESE negative cases.*
Conclusion: *Basal, post clonidine GH levels, has BMI and smoking are predictive factors for fertility potentials, our model have high sensitivity in predicting fertility potentials among positive TESE males. Basal GH can significantly predict TESE negative males.*
Keywords: *fertility potential, TESE*

1. Introduction

Spermatogenesis is the biological process responsible for the generation of sperm cells. During this process, germ cells develop into haploid spermatozoa. Spermatogenesis occurs within the seminiferous tubules, reduced fertility or infertility can occur due to a decrease in the quantity of spermatozoa, changes in their structure, and impaired motility 1.

Dysfunctions can manifest in any of these components, leading to a complete breakdown of the process. These anomalies can result in impaired or diminished spermatogenesis. In more severe cases, azoospermia might occur, resulting in infertility 2.

They establish communication with adjacent Leydig cells, blood vessels, peritubular tissue of the seminiferous tubules, and Sertoli cells. They preserve the trophic factors of these cells and contribute to the control of peristalsis in the seminiferous tubules 3.

The growth factors and neuroendocrine substances affect the ability of myofibroblasts to contract, which is important for the transportation of spermatozoa. In addition, intrinsic factors are crucial in controlling the circulation of blood in the inter tubular microvasculature 4,5.

The control of spermatogenesis is dependent on external inputs originating from the brain and pituitary gland. The pituitary gland awaits a signal from the brain to initiate the secretion of luteinizing hormone (LH). The signal corresponds to the rhythmic release of the hormone gonadotropin-releasing hormone (GnRH), LH prompts the Leydig cells to generate testosterone 6.

Testosterone exerts a substantial impact on the process of spermatogenesis, as well as several other physiological activities in the body. Sertoli cells are activated by follicle-stimulating hormone (FSH), a crucial signal that facilitates the development of germ cells. Sertoli cells release inhibin, which plays a role in the feedback system 4.

Growth hormone (GH) is produced in several organs, including the testes, and serves autocrine and paracrine roles. Several peptides and proteins, including GH, IGF-1, cytokines, activin, inhibin, follistatin, and estrogen regulate spermatogenesis through autocrine and paracrine mechanisms 7.

The growth hormone stimulates sperm production by acting both directly and indirectly through hepatic IGF-1 at the testicular level. GH accelerates the initial growth of spermatogonia and guarantees their full maturity 7.

Men with growth hormone deficiency have testes of reduced size. GH deficiency has been seen in phenotypically normal, azoospermia males who experience maturation arrest. This observation has been verified by clonidine stimulation testing. In contrast, males with GH insufficiency have a low or nonexistent sperm count. Men with GH resistance also have decreased fertility 8-10.

Thus, we conducted a cross section study to assess predictors of fertility potentials and TESE outcomes among adult males including growth and sexual hormones.

2. Materials and methods

We enrolled 162 males who attended the Andrology out-patient clinic, Kasr El Aini hospital, Cairo University. We enrolled patients with age between (20 – 45 years), with good general health and none had received medication for at least 60 days before the study, and Free of varicocele. Patients with family history suggestive of hereditary disorders, history of cancer treated with chemotherapy or radiotherapy, cases of Hypogonadotrophic Hypogonadism, and history of seizures, cardiovascular disease or diabetes were excluded from the current study.

All individuals were subjected to the following steps: medical history taking of personal, marital history, type of infertility, past history of previous operations or history of other diseases that could affect fertility status.

Thorough general examination was performed for secondary sexual characters, any factor that affects overall health which can theoretically be responsible for abnormalities in sperm production and to estimate body mass index. The examination for genital area was performed with the patient in the upright position. The external genitalia were inspected, testes were palpated and were graded as small, moderate, or normal. The epididymis and vas deferens were bilaterally palpated. The patient was asked to perform the Valsalva's manoveur to detect varicocele which when present was graded as small, moderate or large.

At least 2 semen analyses were performed for each included male. They were instructed to bring semen samples by masturbation after sexual abstinence for 3-5 days. Semen analysis was performed according to World Health Organization (WHO) recommendations.

Hormonal assay: Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin (PRL) and Testosterone were measured to exclude cases of hypogonadotrophic hypogonadism, clonidine test, and insulin hypoglycemia induced test.

Microsurgical Testicular sperm extraction (TESE) was carried out under general anaesthesia. Through a small vertical incision in the median scrotal raphe (2 cm), the skin, the dartos muscle and tunica vaginalis were opened to expose the tunica albuginia. The subtunical vessels were identified under the surgical microscope and avoided. A stay suture of 5/0 Prolene was placed into the tunica albuginia, after which a linear transverse (1 cm) incision was made, with care being taken to avoid subtunical vessel injury. The testicular tissues were observed under optical magnification (×24). If no morphologically normal tubules were observed, the incision was extended, and blunt dissection performed between the septa of the testicular parenchyma to expose multiple areas. Copious irrigation

of the field with Ringer's lactate solution was carried out to prevent blood from obscuring the field, and a small sample was taken from the most dilated tubules, examined for the presence of spermatozoa. The sample was placed in Bouin's solution for histopathological evaluation from which we exclude cases of other pathologies. Bipolar diathermy was applied carefully to ensure proper haemostasis. The tunica albugenia was closed using 6/0 Prolene, and the tunica vaginalis was closed with 3/0 Vicryl sutures. If the fresh examination of the minced testicular tissue is negative for sperm. The contralateral testis was exposed for micro-TESE.

We developed a prognostic algorithm for prediction of poor TESE outcomes and fertility potentials. Fertility Score= 4.442 + (Basal GH*0.074) + (GH_CLON*0.035) - (FSH*0.021) (BMI*0.062)-(Smoker*0.429). The net result of this equation should be approximated to the nearest integer to predict the fertility status where, 1=TESE Negative, 2= TESE Positive, 3=Oligozoospermia, and 4= Fertile control.

Statistical methods: The Multivariate Regression Analyses were deployed to detect the role of GH values, together with other independent factors, in prediction of the main outcome measures. Receiver Operator Characteristic (ROC) curve analysis was used to detect the cut off levels for the selected variables. The curve results were not considered when the Area Under the Curve is less than 0.6. P values less than or equal to 0.05 are considered significant. All values are two-tailed unless stated otherwise. Statistical analysis is conducted using SPSS Ver. 26 (IBM SPSS Statistics, Armonk, New York, USA).

3. Results and Discussion

Correlation analysis revealed that potential predictors are the smoking status, testicular volume, BMI, Serum FSH, basal GH, GH after clonidine and after insulin stimulation (Table 1).

Table (1): Correlation analysis on factors affecting fertility potentials:

	Fertility Potential	
	r	P value
Basal GH	0.166*	0.035
Smoker	-0.211-**	0.007
Age	0.093	0.259
Testis Volume	0.194*	0.047
FSH	-0.223-**	0.004
TT	-0.075	0.342
PRL	0.003	0.973
BMI	-0.282-**	<0.001
GH after clonidine	0.238**	0.002
GH after Insulin	0.156*	0.048

Based on the correlation analysis shown in table (1), we analysed the potential predictors (Table 2) in a multivariate regression analysis. None of these selected independent factors were predictive of fertility potential, except basal GH and the BMI.

Table (2): Regression analysis using all the possible predictors of fertility potential

	B	Std Error	Std Beta	T	P	LB-95% CI	UB-95% CI
(Constant)	3.038	1.007		3.018	.003	1.040	5.036
Basal GH	.073	.034	.197	2.110	.037	.004	.141
GH_CLONIDINE	.021	.019	.104	1.107	.271	-.016	.058
GH_INSULIN	-.003	.013	-.024	-.262	.794	-.029	.023

Testis Volume	.074	.046	.150	1.596	.114	-.018	.165
FSH	-.016	.009	-.176	-1.877	.063	-.034	.001
BMI	-.048	.024	-.190	-1.980	.050	-.097	.000
Smoker	-.381	.202	-.176	-1.889	.062	-.781	.019

The second regression analysis showed that smoking, BMI, and FSH correlates negatively with the fertility potential. Moreover, basal GH and GH level after clonidine positively correlate with the fertility potential but GH level after insulin stimulation does not (Table 3).

Table 11: Multi-variant regression analysis including possible predictors after exclusion of the testicular volume

	B	Std Error	Std Beta	T	P	LB-95% CI	UB-95% CI
(Constant)	4.442	.687		6.463	.000	3.084	5.800
Basal GH	.074	.029	.179	2.516	.013*	.016	.131
GH after clonidine	.035	.014	.175	2.426	.016*	.007	.064
GH after insulin	.012	.009	.093	1.298	.196	-.006	.030
FSH	-.021	.008	-.192	-2.699	.008*	-.036	-.006
BMI	-.062	.021	-.213	-2.927	.004*	-.104	-.020
Smoker	-.429	.175	-.177	-2.455	.015*	-.774	-.084

Fertility Score= 4.442 + (Basal GH*0.074) + (GH_CLON*0.035) - (FSH*0.021) (BMI*0.062)-(Smoker*0.429). The net result of this equation should be approximated to the nearest integer to predict the fertility status where, 1=TESE Negative, 2= TESE Positive, 3=Oligozoospermia, and 4= Fertile control.

When applying the model to our cases the equation truly predicts the fertility status in only 38/162 (23.5%) of the cases. The equation works the best in men with positive TESE as it successfully predicted 83% of cases in this group. Comes next, men with oligozoospermia (50%), then the control group 2/50 (4%), and finally the TESE negative group 1/50 (2%).

GH after clonidine stimulation correlates positively with total motile count. Other semen parameters do not correlate with basal GH or GH after insulin or clonidine stimulation (Table 4).

Table (4): Correlation between basal GH, GH after clonidine and Insulin stimulation and the different semen parameters in the oligozoospermia group

		Basal GH	GH_CLON	GH_INSULIN
Sperm count/ml	r	-.236	.230	.305
	P value	.143	.154	.056
Volume	r	-.059	.235	.024
	P value	.715	.144	.883
Total Count	r	-.206	.295	.244
	P value	.203	.065	.130
Motility	r	-.171	.264	.244
	P value	.291	.099	.129
Total Motile Count	r	-.127	.347*	.272
	P value	.436	.028	.090
Abnormal Forms	r	.218	-.343	-.079
	P value	.285	.086	.700

Testis Volume	r	-.063	.122	.051
	P value	.698	.453	.754

There are several factors that could potentially predict positive TESE in men with azoospermia due to spermatogenic arrest. Among the various predictive factors only testicular volume and serum FSH level correlate with TESE outcome.

Table (5) Correlation analysis between TESE outcome and the age, BMI, testicular volume, FSH, TT, PRL, GH [basal, after clonidine and Insulin stimulation]

	TESE	
	R	P value
Age	-0.014	0.916
BMI	-0.140	0.279
Testicular Volume	0.315*	0.020
FSH	-0.251-*	0.049
TT	-0.118	0.361
PRL	-0.039	0.764
Basal GH	0.209	0.104
GH_CLON	-0.121	0.347
GH_INSULIN	-0.029	0.822

Binary logistic regression of testicular volume and serum FSH levels in 62 men with azoospermia due to spermatogenic arrest revealed that only FSH could predict TESE outcome in this group of cases (Table 6).

Table (6): Binary logistic regression analysis including the FSH and Testicular volume as possible predictors surgical sperm retrieval in men with azoospermia

	B	SE	Wald	df	P	OR	LB-95% CI	UB-95% CI
Testicular Volume	.291	.212	1.894	1	0.169	1.338	.884	2.027
FSH	-.268	.134	4.005	1	0.045	.765	.588	.994
Constant	-2.425	2.702	.805	1	0.369	.088		

Receiver Operator Characteristic (ROC) curve analysis is used to detect the cut off levels at which sperm recovery yield change. For the GH assessment only, the basal GH could be applied to predict the SRR in men with azoospermia, AUC=0.672 (95% CI: 0.499 to 0.844) (figure 1). Growth hormone after clonidine (AUC= 0.510) or Insulin stimulation (AUC=0.556) and therefore, cannot differentiate between TESE positive and TESE negative cases.

Testicular volume in 54 cases (missing=8) predicted TESE outcome with an AUC= 0.715 (95% CI: 0.532 to 0.899. Figure 2). The observed cut off level for testicular volume is 10.85 ml, the sensitivity is 75.0% and the specificity is 67.0% (Table 6). Interestingly, the FSH was more powerful in prediction. The AUC= 0.750 (95% CI: 0.613 to 0.887; Figure 11). At FSH level above 5.55 mIU/ml the TESE drops significantly. The sensitivity is 80.0% and the specificity is 67.0% (figure 3).

At basal GH level ≥ 1.4 ng/ml, TESE outcome is more favourable (sensitivity =75.0% and specificity 58.0%; Table 24). At this cut off level the basal GH identified could truly predict successful sperm extraction in 9 out of 12 men with positive TESE (75.0%) and truly predicts no sperm retrieval in 29/50 (58%) of TESE negative cases (Table 25). The cut off level provided overall true prediction in 38 out of 62 cases (61.3%)

Table (7): TESE outcome analysis among men with basal GH above or equal to 1.4 ng/ml and those with GH level below 1.4 ng/ml

Basal GH level/TESE Outcome	N/F	%	True	False
>=1.4 and TESE Positive	9	14.5	Yes	
Less than 1.4 and TESE Positive	3	4.8		Yes
Correct prediction among TESE Positive cases	9/12	75	--	--
Less than 1.4 and TESE Negative	29	46.8	Yes	
>=1.4 and TESE Negative	21	33.9		Yes
Correct prediction among TESE Negative cases	29/50	58	--	--
Total	62	100		
Overall true prediction 38/62 (61.3%)				
Overall false prediction 24/62 (38.7%)				
N= Number, F= Frequency				

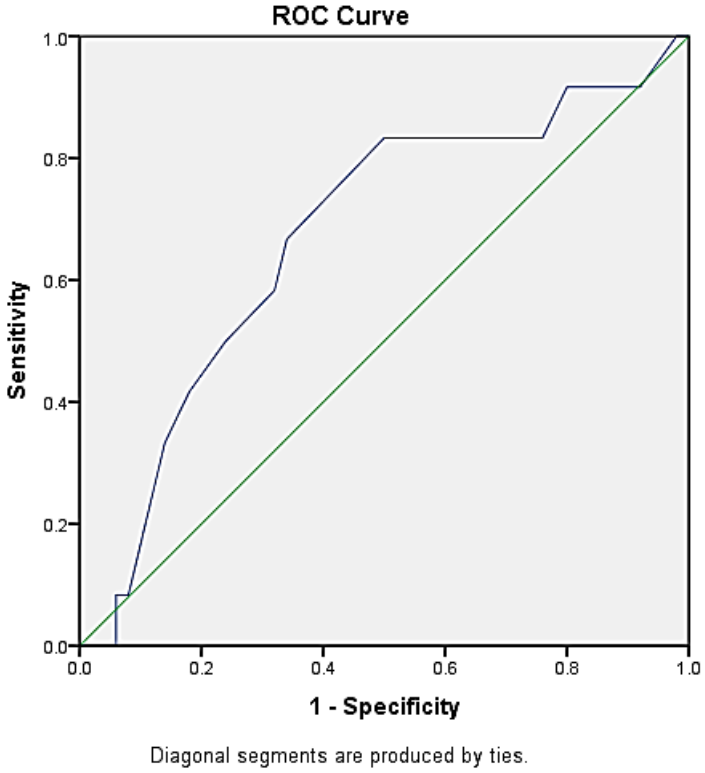

Figure (1): Receiver operating characteristic curve analysis of the basal Growth Hormone assay in 62 men with azoospermia

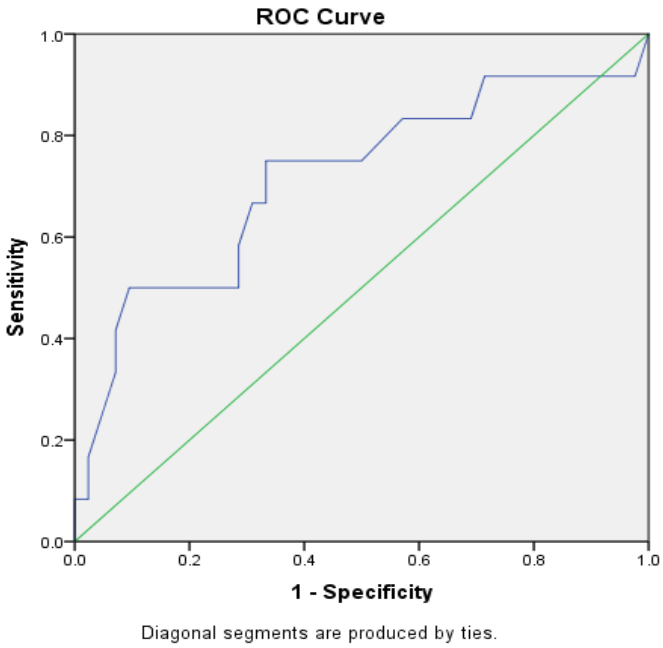

Figure 2: Receiver operating characteristic curve analysis of the Testicular Volume (TV) in 54 men with azoospermia

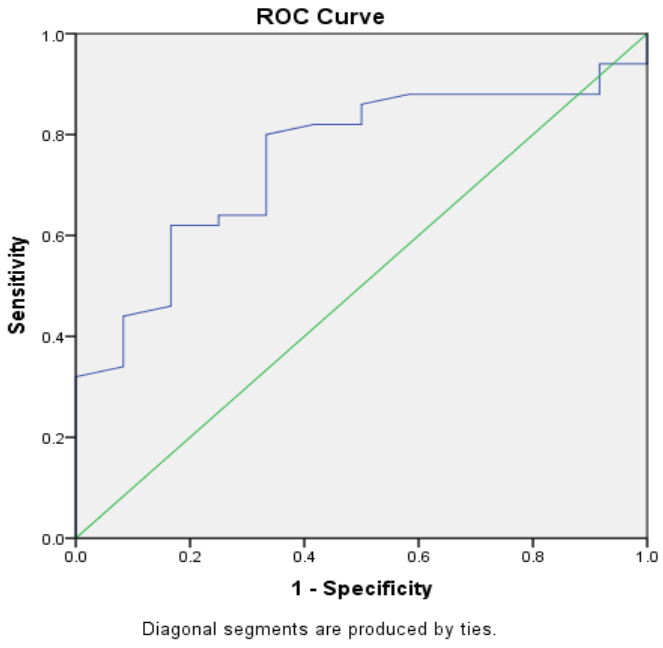

Figure 3: Receiver operating characteristic curve analysis of the FSH in 62 men with azoospermia.

Spermatogenesis is a crucial mechanism for human reproduction. Gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone are all crucial for the growth and maturation of sperm 12.

Much attention has been directed into the impact of growth hormone (GH) on the reproductive system in both males and females. Reproductive dysfunction can occur because of Growth Hormone insufficiency 7.

In the current study, a multivariate regression was deployed, none of these independent factors were predictive of fertility potential, except basal GH and the BMI. Although GH levels significantly increase after Insulin and clonidine stimulation. Insulin seems to be a stronger stimulant of GH Secretion. However, multivariate regression analysis revealed that clonidine but not insulin predicts fertility potential.

Fraser and colleagues supported our findings and reported that "In those without growth hormone deficiency, clonidine provoked a significantly higher mean plasma growth hormone response and gave fewer false subnormal responses (apparent growth hormone deficiency) than insulin" 8. Also, Darshana

and her colleagues supported our findings as they found that 60 min after clonidine stimulation was the best single sample to rule out GHD in adults with high specificity 13.

We found that GH after clonidine stimulation correlates positively with total motile count. Other semen parameters do not correlate with basal GH or GH after insulin or clonidine stimulation.

Karla and co-workers in their open labelled, non-randomized study on 14 men with oligo-astheno-zoospermia supported our finding, when the patients responded to 1.5 IU of GH per day for six months and observed improvement in semen volume, count and great improvement in motility, and natural conception in 3 couples. Although our observation is limited to total motile count, our study was non-interventional and based on observation only. Improvement in the other semen parameters would have been expected if the treatment continues for 6 months as Karla and her colleagues did 14.

We found that fertility potential of a given patient can be calculated using the following equation: Fertility Score= 4.442 + (Basal GH*0.074) + (GH_CLON*0.035) - (FSH*0.021) -(BMI*0.062)-(Smoker*0.429). The net result of this equation should be approximated to the nearest integer to predict the fertility status where, 1=TESE Negative, 2= TESE Positive, 3=Oligozoospermia, and 4= Fertile control. Using our prediction model accurately predicted the fertility status in 38/162 (23.5%) cases. However, the equation works the best in men with positive TESE as it successfully predicted 83% of cases in this group. Comes next, men with oligozoospermia (50%), then the control group 2/50 (4%), and finally the TESE negative group 1/50 (2%).

The cumulative success in testing azoospermia for successful TESE would be 11/62 (18%). Up to our knowledge no published articles on this topic or other investigators that have conducted similar work before. We observed several factors that could potentially predict positive TESE in men with azoospermia due to spermatogenic arrest. Among the various predictive factors only testicular volume (TV) and serum FSH level correlate with TESE outcome.

Logistic regression of testicular volume and serum FSH levels in 62 men with azoospermia due to spermatogenic arrest revealed that only FSH could predict TESE outcome in this group and our results come in agreement with Ghalayini et al., who found that patients with NOA and lower FSH levels had higher Sperm Recovery Rate (SRR) with cut off value 24 mIu / ml. This cut off value may differ from our findings as cases in Ghalayini study includes all pathological conditions including hypo-spermatogensis, maturation arrest, KF cases and sertoli cell only syndrome while our study is done on maturation arrest only 15.

Also, Colpi and colleagues showed that mean FSH level was significantly higher in the TESE negative group 16. Qi and his colleagues found plasma FSH level to predict SRR with best cutoff value less than 19.01 for predicting successful sperm retrieval in men with NOA 17.

Corona and Colleague reported that age did not represent a limiting factor for undergoing TESE in NOA and this come in agreement with our findings. They also reported that a mean TV higher than 12.5 ml predicted SRR in > 60% with an accuracy of 86.2% 18.

In our results, TV correlated positively with the success rate of-TESE, which come in agreement with Kizilkan and his team 19. It's role as a good predictor variable remains inconsistent, in a meta-analysis of 1764 cases, reported no threshold of TV associated with the SRR 20.

However, Corona and his coworkers in their metaanalysis including 117 selected articles enrolling 21, 404 patients reached a cut point of 12.5 ml. They found out that testicular volume higher than 12.5 ml predicted SRR in > 60% with an accuracy of 86.2% 18.

In the current study the observed cut off level for testicular volume is 10.85 ml, the sensitivity is 75.0% and the specificity is 67.0%. Interestingly, the FSH was more powerful in prediction with a wider AUC. At FSH level above 5.55 mIU/ml the TESE drops significantly. The sensitivity is 80.0% and the specificity is 67.0%. Some investigators proposed that serum FSH can predict positive SRR after Conventional TESE 21; however, these results were contradicted by other authors 22,23.

Serum FSH was a good predictor since we included men with maturation arrest. This is well supported by Silber and his colleagues who reported that serum FSH levels inversely correlated with the number of germ cells in the testis but not with more advanced stages of spermatogenesis 24.

Perhaps the presence of round, late spermatids, and mature spermatozoa in positive TESE cases have a substantial impact in our analysis. Similar results were reported by Li et al., in a meta-analysis of studies using only microsurgical TESE, and the same study documented that FSH had a stronger predictive value in men from East Asia 17.

Corona and his coworkers supported this finding as they also found out that SRR was lower in studies performed in men from East Asia and the Arabian Peninsula when compared to Europe and North America. Whether this finding is attributed to ethnicity factor or to superior surgical facilities, refined skills and better techniques are still questionable 18

Receiver Operator Characteristic (ROC) curve analysis is used to detect the cut off levels at which sperm recovery yield change. For the GH assessment only, the basal GH could be applied to predict the SRR in men with azoospermia, Growth hormone after clonidine or Insulin stimulation cannot differentiate between TESE positive and TESE negative cases.

We found that, the basal GH at a cut off level (1.4 ng/ml) could truly predict successful sperm extraction in 9 out of 12 men with positive TESE (75.0%) and truly predicts no sperm retrieval in 30/50 (60%) of TESE negative cases. The cut off level provided overall true prediction in 38 out of 62 cases (61.3%). Up to our knowledge, no previous studies reached this conclusion.

This came in agreement with Magon and coworkers who claimed that GH is deficient in phenotypically normal azoospermic men with maturation arrest, conversely, the sperm count is low or nil in men with GH deficiency 7. GH resistance in men is also associated with reduced fertility 7,10

4. Conclusion

We finally concluded that Basal, post clonidine GH levels, has BMI and smoking are predictive factors for fertility potentials, our model have high sensitivity in predicting fertility potentials among positive TESE males. Basal GH can significantly predict TESE negative males.

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