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USE OF MESENCHYMAL STEM CELLS IN REPAIRING MALE INFERTILITY SUFFERING FROM OLIGOSPERMIA.

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ABSTRACT

Germ cells must develop along distinct male paths to produce spermatozoa required for sexual reproduction. We show the expression of early germ cell markers cd105 in these cells. Mesenchymal stem cells (MSCs) are multipotent stem cells with abundant sources, active proliferation, and multidirectional differentiation potential. MSCs play a role through cell homing, secretion of active factors, and participation in immune regulation. Our findings provide direct evidence that human bone marrow cells and umbilical cord can differentiate to putative male germ cells and can provide a potential source of male germ cells that could sustain sperm production. This article summarizes the current research progress on mesenchymal stem cells in diseases related to infertility; advancements indicate that this emerging evidence contributes to solving problems related to male infertility. Furthermore, these data indicate the potential to harness the properties of stem cells for clinical applications and are also solve the problem of oligospermia persons and giving them absolve to become a normal person. According to count, before the injection the total number of cases were less than 15 million (100%), the mean was 6.04 ± 5.33 and after the injection of mesenchymal stem cells 8 cases (32%) were still less than 15 million but 17 cases (68%) were become more than 15 million, the mean was 23.02 ± 13.64, so p-value was <0.001 which is significant.

Furthermore, According to vitality, before injection the minimum and maximum percent were (2%,28%, respectively), and also the mean was 13.0 ± 7.27. After the injection the minimum and maximum percent of vitality was (7,83%), the mean was 50.15 ± 25.47. Moreover, the p-value is less than 0.001, which is significant.

Conclusively*,the results from recent preclinical studies regarding stem cell-based therapies are promising. Stem cell-based therapies cast a new hope for infertility treatment as a replacement or regeneration strategy.*

Keywords: Infertility**,** Mesenchymal stem cells**, oligospermia ,** Stem cell

INTRODUCTION

Infertility is a global reproductive disorder that is caused by a variety of complex diseases. Infertility affects the individual, family, and community through psychological, social and economic consequences. Infertility is a common and complex disease characterized by the failure to establish a clinical pregnancy after 12 months of regular and unprotected sexual intercourse *(Liang et al.,2020).* The main features and application prospects of mesenchymal stem cells in the future of infertility should be understood by clinicians. Mesenchymal stem cells (MSCs) are multipotent stem cells with abundant sources, active proliferation, and multidirectional differentiation potential. MSCs play a role through cell homing, secretion of active factors, and participation in immune regulation. Another advantage is the factors involved in the application of MSCs (He *et al.*,2020).

According to the latest statistics, infertility is estimated to affect 9% to 18% of reproductive-aged couples worldwide, and the incidence is increasing each year (Aghajanova *et al.*,2017). Oligospermia is a male fertility issue characterized by a low sperm count. This includes the ability to get and maintain an erection, as well as produce ejaculation at orgasm. It is known that DNA damage occurs in sperm due to various reasons. The most common cause of DNA damage in sperm is oxidative stress. Oxidative stress occurs due to the imbalance between the production of reactive oxygen species and the antioxidant defense system (Dorostghoal *et al.*,2017)

The fact that the spermatozoon membrane contains high amounts of polyunsaturated fatty acid and its cytoplasm has an inadequate antioxidant capacity makes spermatozoa highly vulnerable to the attacks of reactive oxygen species and lipid peroxidation *(*Vernet *et al* .,2004*)*. For this reason, many different factors can cause oxidative stress and DNA damage in spermatozoa. For example, it was reported that heat stress, (Banks *et al.*,2005) cryopreservation of sperm (Baumber *et al.*, 2003) and chilling of sperm (Hansen *et al.*,2005) cause DNA damage in the sperm. It is known that smoking, cancer therapies, varicocele, and cancer can lead to DNA

damage in the human sperm (Schulte *et al* .,2010). Also, DNA damage can occur in germ cells during spermatogenesis and some DNA damaged germ cells are eliminated via apoptosis (Paul *et al.*,2008).

Therefore, the disturbances related to the apoptotic mechanism might lead to DNA damaged sperm production.

MATERIALS & METHODS

Umbilical Cord Blood Collection

Umbilical cord blood samples (n =20) were collected in 50 ml Costar tubes from delivering women at (37- 40 weeks) were collected from the Department of Obstetrics and Gynecology, Faculty of Medicine Hospitals at Menoufiya University, Egypt. Umbilical cord blood samples were obtained from human healthy pregnant women after giving birth as fast as possible to preserve cell viability and the blood samples were processed by the Stem Cells Laboratory at Genetic Engineering and Biotechnology Research Institute (GEBRI), the University of Sadat City according to the esthetical committee roles. The study started at January 2018 and ended in June 2020. The volunteers' age ranged from twenty-five to thirty. They were not suffering from any chronic diseases and not taking any medications. UCB was collected from the umbilical cord vein with the informed consent of the mother. A tube system containing 5 mL of citrate phosphate dextrose anticoagulant was used. All UCB samples were processed within 6h after deliveries**.**

1-Preparation of CD^{105} Mesenchymal Stem Cells

1.2.Mononuclar Cells Separation

1.3.Immuno-magnetic labeling and separation of CD^{105+}/ $^{CD105-}$ Progenitor Stem Cells

1.4. Magnetic labeling of CD^{105+} and Separation with auto MACS Separator (Miltiny Biotech Germany).

1.5. CD^{105+} Mesenchymal StemCells Proliferation.

1.6. Extraction of CD^{105+} Cells.

2. MCF-7 Cell Line

3. MTT Cell Viability Assay

4. Quantitative RT-PCR of Bax, Bcl2,p53 and Casp 9, Gene exepression:

5. Cell Cycle analysis

6. Apoptosis study by Annexin V assay Kits *(Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA*).

RESULTS AND DISCUSSION

Infertility is one of the commonest problems that affects 13-18% of couples with increasing male reproductive problems. There is a male factor that involves up to 50% of all infertile couples **(Geijen *et al*.,2004)**. the infertile male presented with abnormal sperm production such as oligospermia in about 11%. Up till now the treatment for male infertility is limited. Studies showed that stem cells can provide a true therapy for these problems. In our study the number of cases was 25. according to demographic data.

***Age**:*

The number of cases less than 40 years old was 18 (72%) and patient higher than 40 years old were 7 (28%), the mean was 36.28 ± 4.26. Men younger than 40 have a better chance of fathering a child than those older than 40. The quality of the sperm men produce seems to decline as they get older. Most men make millions of new sperm every day, but men older than 40 have fewer healthy sperm than younger men. The amount of semen and sperm motility decreases continually between the ages of 20 and 80.

***Duration of marriage**:*

The minimum duration of marriage was 2 years, the maximum duration was 13 years so the mean was 6.24 and the standard deviation was 3.15.

***Occupation**:*

In the present study, the cases are divided into three-part. the first part the patient worked light work was about 14 patients (56%), the second part the patient worked heavy work were about 7 patients (28%) and the third part the patient worked thermal work were about 4 patients (16%). Farmers and painters/varnishers showed a significantly higher proportion of reduced sperm counts in addition, significantly more farmers presented with a history of male descended testes than other occupational groups Metalworkers/welders formed significantly higher proportions of patients with reduced sperm motility The relatively poor semen parameters of the painters/varnishers could be caused by exposure to toxins. This may also apply to the farmers (fertilizers, herbicides); however, the elevated rate of maldescended testes suggests an effect of exposure during prenatal development or a genetic cause. The findings for metalworkers/welders maybe because of heat or toxins at the workplace (Cherry *et al.,* 2008).

Body mass index:

In this study when we done body mass index found that the minimum number was 21.2 kg/m2 , the maximum number was 37.0kg/m2, the mean was 171.16 ± 5.28 This was going with a study done by Androl. (2017). A high BMI is associated with negative effects on sperm quality. Overweight and obese men are more likely to have abnormally low sperm concentrations, total sperm count, total motile sperm count, the relative amount of type A motility, and relative amount of progressive motility (A + B) than men with normal weight. Further research is required to evaluate the relationship between male BMI and sperm quality in larger populations.

Table (1): Distribution of the studied cases according to demographic data (n = 25)

Demographic data	**No.**	**%**
Age (years)		
<40	18	72.0
≥40	7	28.0
Min. – Max.	29.0 – 43.0	
Mean ± SD.	36.28 ± 4.26	
Median (IQR)	36.0 (33.0 – 40.0)	
Duration of marriage (years)		
Min. – Max.	2.0 – 13.0	
Mean ± SD.	6.24 ± 3.15	
Median (IQR)	6.0 (3.0 – 8.0)	
Occupation		
Light work	14	56.0
Heavy work	7	28.0
Thermal related work	4	16.0
Body weight (kg)		
Min. – Max.	65.0 – 103.0	
Mean ± SD.	75.0 ± 8.77	
Median (IQR)	71.0 (69.0 – 79.0)	
Height (cm)		
Min. – Max.	163.0 – 180.0	
Mean ± SD.	171.16 ± 5.28	
Median (IQR)	169.0 (167.0 – 177.0)	
BMI (kg/m^2)		
Min. – Max.	21.22 – 37.38	
Mean ± SD.	25.62 ± 3.01	
Median (IQR)	24.84(24.38 – 26.23)	

SD: **Standard deviation**

The present results, also made the distribution of studies according to medical data.

***According to parity*:** About 23 patients (92%) were primary infertility but only 2 patients (8%) were secondary infertility.

***According to the history of chronic disease*:** About 21 patients (84%) were - non-history of disease, 2 patients (8%) were diabetic and 2 patients (8%) were hypertension this is going with the study of World J Mens Health. (2017).

The studies discussed in this review demonstrate that glucose metabolism is of great importance for sperm cells, either type 1 diabetes or type 2 diabetes could have detrimental effects on male fertility, especially on sperm quality, such as sperm motility, sperm DNA integrity, and ingredients of seminal plasma. Diabetes may influence the epigenetic modification during sperm spermatogenesis and that these epigenetic dysregulations may be inherited through the male germline and passed onto more than one generation, which in turn may increase the risk of diabetes in offspring.

The dramatic increase in diabetes, one of the human metabolic disorders warrants considering the influence of environmental factors on the germline. Parental nutrition and metabolism are critical determinants of adult offspring health, maternal hyperglycemia and trans generational epigenetic inheritance may be worthy of consideration. Also, the study 0f hypertension is going with World J. Mens Health(2017).

The studies discussed in this review demonstrate hypertension and Existing data suggest an association between hypertension and impaired semen quality. Men diagnosed with hypertension have a lower semen volume, sperm motility, total sperm count, and motile sperm count relative to men in the cohort who did not carry a diagnosis of hypertension. Importantly, more men with a diagnosis of hypertension had impaired semen volume, concentration, and total motile count, according to WHO 5th edition criteria for subfertile semen parameters. Moreover, the use of beta-blockers was associated with lower semen volume, concentration, motility, total sperm count, and total motile sperm count, while men taking other antihypertensives had more isolated impairments in semen parameters.

According to the history of surgical operation:

The present results had about 23 patients (92%) who made any operation, 2 patients (8%) were mad varicocele operation. This is going with the study of Advances in Urology / 2012 / Article).

Its epidemiologic features suggest that it is a progressive pathology with a genetic predisposition. Recent studies on the physiopathology of varicocele-related infertility have shown the likely influence of ultrastructural testicular changes and increased oxidative stress with implications on the seminal antioxidant capacity and sperm chromatin integrity. Controversy remains regarding the benefit of varicocele repair to improve male fertility. Evidence exist both in favor and against it, but as of now, most specialty societies recognize that varicocele is detrimental to male reproductive health and its treatment may improve sperm function and chances of conceiving.

***According to smoking*:** About 13 patients (52%) were nonsmoker, 4 patients (16%) were moderate smokers and 5 patients (20%) were heavy smoke. Confirm that a large volume of retrospective data exists examining smoking and the effects on semen analysis parameters and IVF outcomes, large-scale, population-wide studies on the effects of smoking on natural pregnancies are lacking. Nevertheless, the majority of the evidence points to the fact that men with infertility, or those having difficulty conceiving, should quit smoking to optimize their chances for successful conception (Jason *et al*., 2015).

Table (2): Distribution of the studied cases according to medical data (n = 25)

Medical data	No.	%
Parity		
1^{ry} infertility	23	92.0
2^{ry} infertility	2	8.0
History of chronic disease		
Non	21	84.0
Diabetic	2	8.0
Hypertension	2	8.0
History of surgical operation		
Non	23	92.0
Varicocele	2	8.0
Smoking		
Non smoker	13	52.0
Smoker	**12**	**48.0**
Mild	3	12.0
Moderate	4	16.0
Heavy	5	20.0

Regarding hormonal profile: Hormones different after injection of stem cells as follow :

Regarding follicle-stimulating hormone (fsh):In this study the FSH of the studied men ranged from 1.30-191 Iu/l , the mean of FSH before injection was 13.01 ± 8.21 , after injection, the mean was 9.61 ± 4.68 so P- value was 0.026 which is significant.

Regarding luteinizing hormone (lh): In this study the LH hormone of the studied men ranged from 1.42-15.4 IU/L , the mean of LH before injection was 9.06 ± 4.76 , after injection the mean was 7.0 ± 3.41 so p value was 0.002, which is significant.

Regarding prolactin hormone (PRL):In this study the PRL hormone of the studied men ranged from 0 – 20 ng/ml ,the mean of PRL before injection was 10.33 ± 6.38 , after injection, the mean was 9.55 ± 4.75 so P- value was 0.796 which is non- significant.

Regarding estradiol hormone (E2):In our study the E2 hormone of the studied men ranged from 10 – 40 pg/ml , the mean of E2 before injection was 38.05 ± 17.93 , after injection the mean was 32.80 ± 10.47 so p value was 0.036, which is significant.

Regarding testosterone hormone : In our study the testosterone hormone of the studied men ranged from 1.3-9.2 pg/ml , the mean of the studied men before injection was 5.67 ± 4.15 , after injection the mean was 6.0 ± 2.61. So P- value was 0.010 which is significant.

P- value for comparing between hormonal profile before and after stem cell injection statistically significant p value < 0.05 and this is confirmed by the study of World J Stem Cells (2016).

Multiple stem cell therapies to restore the androgenic function of the testes are under investigation . Leydig cells derived from bone marrow, adipose tissue, umbilical cord, and the testes have shown promise in future therapy for primary hypogonadism. The results demonstrated that the murine bone marrow cells had the potential to differentiate into germ cells, Sertoli, and Leydig cells *in vivo*. However, it was unknown which precursor cell from the bone marrow differentiated into each end testicular cell type.***(*** LO *et al* , 2003) demonstrated that murine testicular stem cells, isolated from the interstitial space of the testis and transplanted into the interstitial space of LH receptor, yielded a time-dependent production of testosterone in a hypogonadal murine model. Yet, these cells were derived from a side population and contained stem cells of multiple lineages including

Table (3): Comparison between hormonal profile before and after stem cell injection

Hormonal profile	Before injection (n = 25)	After injection (n = 25)	Test of Sig.	P
FSH hormone(IU\L)				
Min. – Max.	0.80 – 30.60	1.20 – 19.90	Z= 2.227*	0.026*
Mean ± SD.	13.01 ± 8.21	9.61 ± 4.68		
Median (IQR)	**12.90 (8.60 – 17.2)**	**11.10 (8.90 – 12.0)**		
LH hormone (IU\L)				
Min. – Max.	1.90 – 18.50	1.40 – 16.0	Z= 3.065*	0.002*
Mean ± SD.	9.06 ± 4.76	7.0 ± 3.41		
Median (IQR)	**9.10 (4.10 – 11.80)**	**7.60 (4.10 – 8.60)**		
PRL hormone (ng/ml)				
Min. – Max.	2.0 – 19.80	2.90 – 16.20	Z= 0.259	0.796
Mean ± SD.	10.33 ± 6.38	9.55 ± 4.75		
Median (IQR)	**9.10 (4.10 – 17.10)**	**9.90 (5.90 – 14.50)**		
E2 hormone (pg/ml)				
Min. – Max.	4.80 – 80.50	8.90 – 50.0	t= 2.222*	0.036*
Mean ± SD.	38.05 ± 17.93	32.80 ± 10.47		
Median (IQR)	**40.0 (27.0 – 44.0)**	**37.0 (29.90 – 40.0)**		
Testosterone hormone (ng/dl)				
Min. – Max.	0.17 – 18.20	2.50 – 10.80	Z= 2.585*	0.010*
Mean ± SD.	5.67 ± 4.15	6.0 ± 2.61		
Median (IQR)	**4.40 (2.90 – 8.80)**	**5.50 (3.90 – 8.10)**		

IQR: Inter quartile range SD: Standard deviation
t: Paired t-test Z: Wilcoxon signed ranks test
P: P- value for comparing between hormonal profile before and after stem cell injection
*: Statistically significant at $P \leq 0.05$.

spermatogonial stem cells, SLCs, and possibly myoid stem cells. As in the previous study, it was difficult to determine, which cell lineage led to the end testosterone-secreting cell.

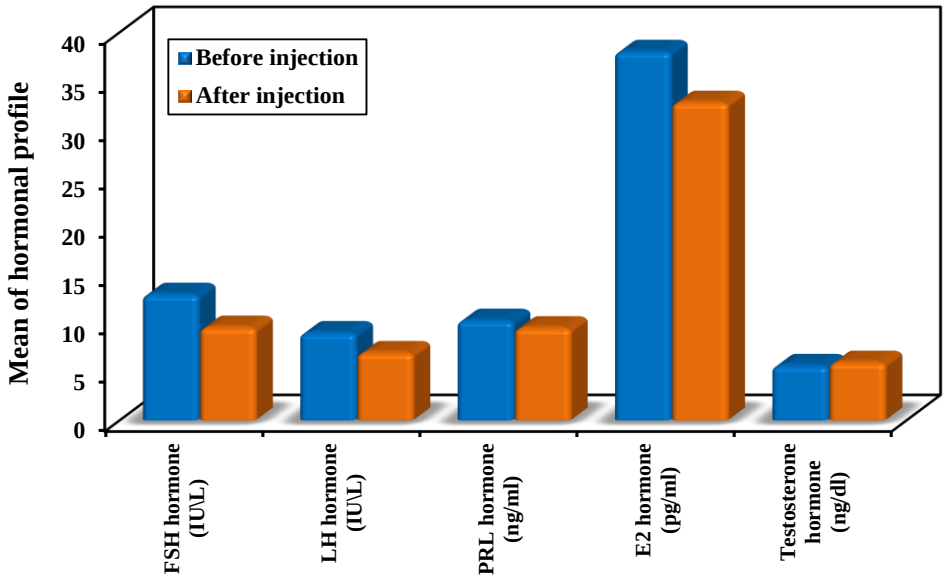

Figure (1): Comparison between hormonal profile before and after stem cell injection

As regards the results of injection of stem cells into two testes the present study:

- ***Regarding semen analysis****:*

  According to count:. *Before* injection the total number of cases was less than 15 million (100%), the mean was 6.04 ± 5.33. after injection of mesenchymal stem cells, 8 cases(32%) were still less than 15 million but 17 cases *(68%)* were becoming more than 15 million, the mean was 23.02 ± 13.64, so P-value was P<0.001, which is very significant.

  According to vitality: .Before injection, the minimum percent was (2%) and the maximum percent was (28%) , the mean was 13.0 ± 7.27. after injection, the minimum percent of vitality was (7%) and the maximum percent was (83%), the mean was 50.15 ± 25.47. so p-value is less than 0.001, which is significant.

  According to abnormal forms: The minimum percent of abnormal forms were (88%) and the maximum percent were (98%), the mean was 93.96 ± 3.18. after injection the minimum percent (81%) and maximum percent were (98%), the mean was 50.15 ± 25.47. so *p*-value for comparing between semen analysis before and after stem cell injection is statistically significant at P < 0.05.

Table (4): Comparison between semen analysis before and after stem cell injection

Semen analysis	Before injection (n = 25)		After injection (n = 25)		Test of Sig.	P
	No.	%	No.	%		
Count (×10^6)						
<15	25	100.0	8	32.0	Z=	^{McN}p
>15	0	0.0	17	68.0	25.758*	<0.001*
Min. – Max.	0.09 – 14.40		0.20 – 44.0		Z=	
Mean ± SD.	6.04 ± 5.33		23.02 ± 13.64		4.319*	<0.001*
Median (IQR)	**3.90(0.90 – 10.1)**		**22.0(11.80 – 33.0)**			
Vitality (%)						
Min. – Max.	2.0 – 28.0		7.0 – 83.0		Z=	
Mean ± SD.	13.0 ± 7.27		50.15 ± 25.47		4.145*	<0.001*
Median (IQR)	**12.0 (8.0 – 18.0)**		**61.0 (18.0 – 67.0)**			
Abnormal forms (%)						
Min. – Max.	88.0 – 98.0		81.0 – 98.0		t=	
Mean ± SD.	93.96 ± 3.18		89.64 ± 5.38		5.048*	<0.001*
Median (IQR)	**94.0 (92.0 – 97.0)**		**90.0 (87.0 – 95.0)**			

IQR: Inter quartile range, SD: Standard deviation, χ^2: Chi square test, McN: Mc Nemar test, Paired t-test, Z: Wilcoxon signed ranks test, P:P- value for comparing between semen analysis before and after stem cell injection

*: Statistically significant at P ≤ 0.05.

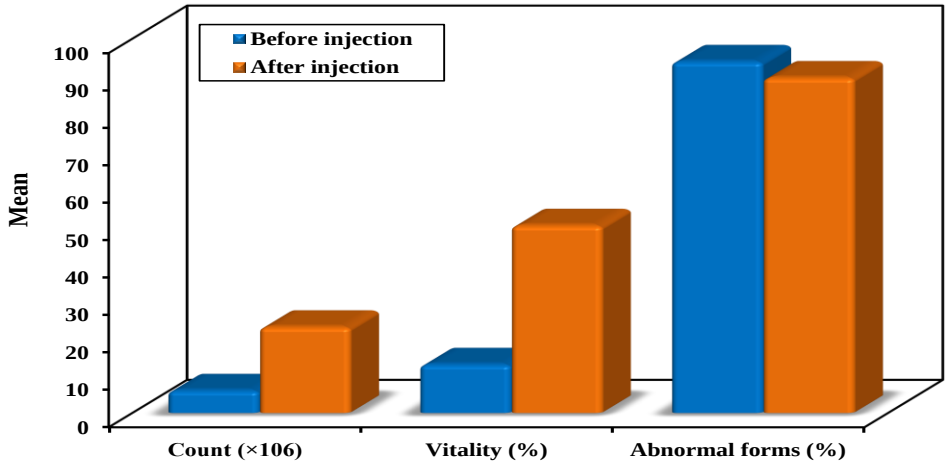

Figure (2): Comparison between semen analysis before and after stem cell injection

In Conclusion:

Infertility is both a medical and a social problem that affects a large male population worldwide. It is associated with a number of pathophysiological conditions, and its pathogenesis is frequently undefined, with relative uncertainty in establishing appropriate treatment choices. In recent years, with the development of research on stem cells, increasing evidence has shown that stem cells, especially mesenchymal stem cells, may become a potential tool for the treatment of infertility. Stem cell-based therapy, as a modality of regenerative medicine, is considered one of the most promising disciplines in modern science and medicine.

This article summarizes the current research progress on mesenchymal stem cells in diseases related to infertility; advancements indicate that this emerging evidence contributes to solving problems related to male infertility. Furthermore, these data indicate the potential to harness the properties of stem cells for clinical applications and are anormal persons. Although remarkable achievements have been made, more also solve the problem of oligospermic persons and giving them asolve to become research support and improvement are needed.

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استخدام الخلايا الجذعية اللحمية في علاج العقم عند الذكور الذين يعانون من قلة النطاف.

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يعتبر العقم من المشاكل الاكثر شيوعا التي تؤثر علي 13-18 % بين الازواج وغالبا ماتكون مشاكل خاصه بعقم الرجال. يتميز الفرد المصاب بالعقم بوجود حيوانات منويه قليله العدد ذات حيويه وحركه ضعيفه وهي تمثل 11-13% . حتي الان فان علاج عقم الرجال محدود للغايه ومعظمهم يخضعون للاخصاب المعملي او الحقن المجهري. تمثل الخلايا الجذعيه علاجا حقيقيا لحل تلك المشاكل حيث وجد ان حقن الخلايا الجذعيه يمكن ان تنتج خلايا جرثوميه في مزارع الخليه كما وجد ايضا قدرته علي تكوين بويضات عند حقنها في المبيض.ومع ذالك يعتبر استخدام الخلايا الجذعيه في علاج العقم محدود للغايه .

الغرض من البحث

استهدفت الدراسه الحاليه تقييم استخدام الخلايا الجذعيه الميزنشيميه في اصلاح العقم في الحالات التي تعاني من قله وعدم كفائه الحيوانات المنويه.

خطوات البحث

ستجري الدراسه الحاليه في وحده عقم الرجال في مركز الاخصاب المساعد بمستشفي الحسين الجامعي جامعه الازهر بالقاهره بالتعاون مع معمل الخلايا الجذعيه في معهد الهنسه الوراثيه والتكنولوجيا الحيويه جامعه مدينه السادات مع مراعاه اخذ موافقه لجنة الاخلاقيات في كليه الطب جامعة الازهر بالقاهرة .

صممت الدراسة لتتناول خمسون فردا من الذكور خمسه وعشرون منهم من الاصحاء وهم مجموعه طبيعيه تماما تم فحصهم داخل وحدة الذكوره ويمثلون المجموعه الضابطة والخمسه وعشرون الاخاري مصابين بالعقم نتيجه قله الحيوانات المنويه وقله حيويتها سيتم تجميع الحالات من المرضي المترددين علي وحده العقم بمستشفي الحسين الجامعي ويترواح اعمارم من 30-40 عاما .

سيتم اخضاع كل الاحالات سواء كانو اصحاء ام مرضي بقله وعدم كفاءة الحيوانات المنويه لفحص الحيوانات المنويه من حيث العدد والحيويه والكفاءة بالاضافه الي فحص هرمونات الذكوره .

سيتم فصل الخلايا الجذعيه من دم النخاع العظمي للمصابين او من دم الحبل السري واكثارها معمليا بالاضافه الي اختبار حيويتها ونقاوتها . سيتم حقن المرضي بالخلايا الجذعيه الميزنشيميه في الخصيتين بعدها يتم متابعة المرضي بعد شهرين – اربعه اشهر وستة اشهر علي مستوي التحليل الهرموني وتحليل السائل المنوي.

التوصية:

باستخدام الخلايا الجذعيه الميزنشيميه ظهور تحسن واضح علي مستوي التحليل الهرموني وتحليل السائل المنوي.