AIM OF THE WORK

The aim of this work was to study a cumulative embryo score system (CES) for the prediction of pregnancy outcome among cases undergoing intracytoplasmic sperm injection (ICSI). Primary outcome was the evaluation of embryo score and the secondary was occurrence of pregnancy; as detected by ultrasonography or a positive serum β-HCG.

PATIENTS

The current study included 100 patients attended to El-shatby maternity university hospital in Alexandria, Egypt.

Inclusion criteria: may be

1. Male factor infertility
2. Cervical factor
3. Absent or Damaged Fallopian Tubes
4. Endometriosis (diagnosed by laparoscopy or U/S)
5. Unexplained Infertility
6. Recurrent Intrauterine Insemination Failure
7. Tubal and Pelvic Adhesions

Exclusion criteria:

1. Poor responders.
2. Previous ICSI failure.
3. Uterine anomalies.
4. Age > 40 years.
5. Genetic diseases.

METHODS

- Consent was obtained from all patients to participate in the current work.
- All patients were subject to the following:
  1. Full history taking.
  2. Full clinical examination
  3. Preparation of semen: Semen parameters were considered to be impaired when the sperm concentration was < 15 x 10^6ml, the progressive motility < 40%, or a normal morphology was exhibited by < 4% of the spermatozoa. The concentration of the assessed sperm suspension was adjusted to 1-1.5 x 10^6/ml, when necessary, by the addition of HTF medium, and subsequently incubated at 37 C in a gas atmosphere of 5% CO2 in air until utilization for ICSI. The specimen was examined microscopically, and at least 100-200 spermatozoa were categorized. Cases of azoospermia or severe oligoasthenozoospermia, sperm was either recovered from the ejaculate by using special sperm preparation techniques such as multiple ejaculation, resuspension and centrifugation or from testes themselves, by using multiple biopsy techniques that increase the likelihood of finding normal foci (TESE).

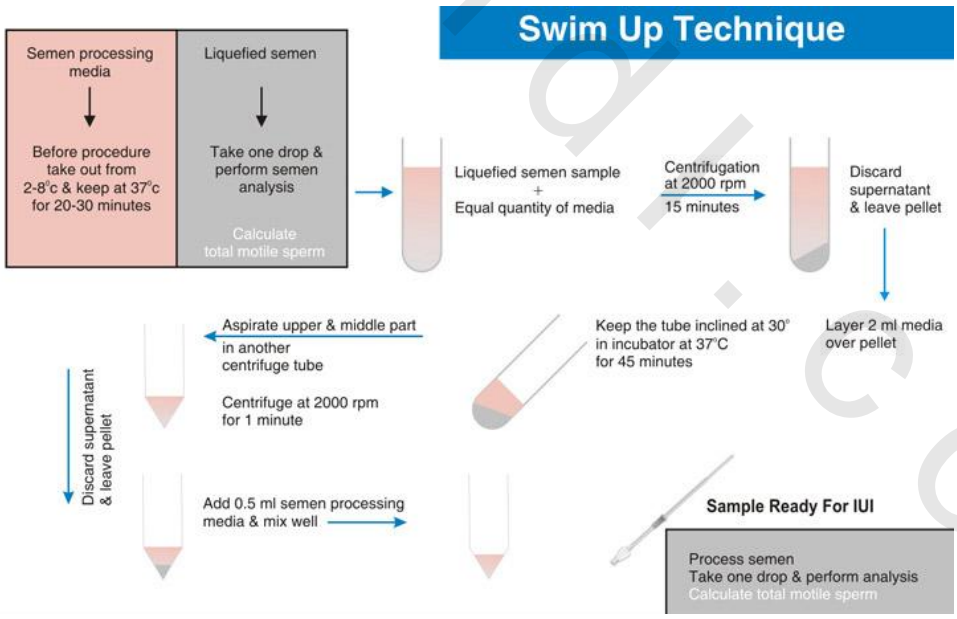


Figure (3): Semen preparation using swim up technique.[85]

4. Controlled ovarian hyperstimulation: Using the Long-GnRH-agonist Protocol, With this regimen, pituitary desensitization was induced by Decapeptyl ® (Ferring) subcutanous administration in

mid-luteal phase of the cycle preceding the planned IVF. Once desensitization was obtained, ovarian stimulation with gonadotrophins (FSH): Fostimon 75® (IBSA) was started at day 2 of the cycle mostly 2 ampules (there's dose adjustment according to each patient) and Decapeptyl injection was continued until human chorionic gonadotrophin (hCG): 2 ampule of Choriomon 5000® (IBSA) was administrated I.M. when leading follicles by U/S reached 18mm.

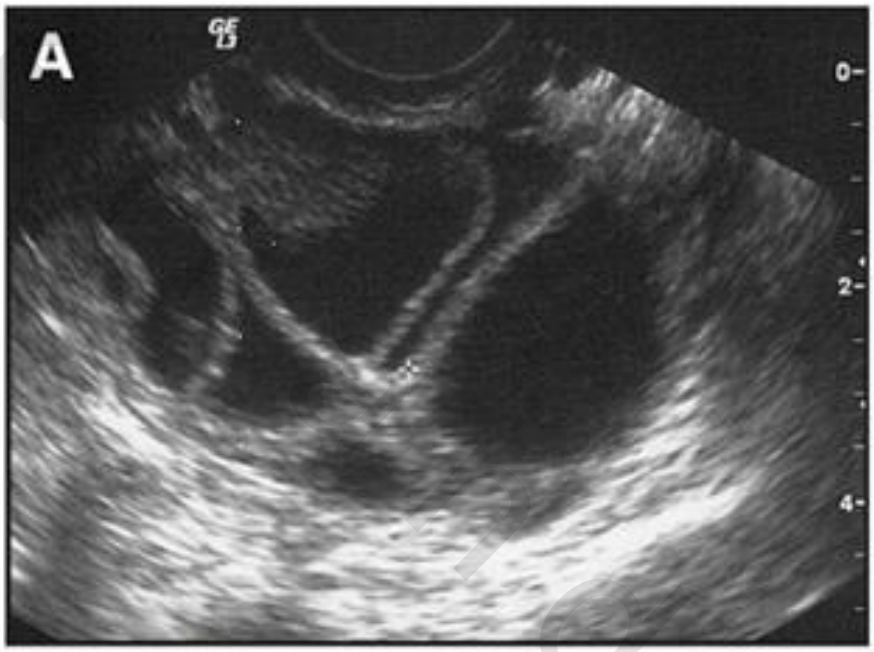


Figure (4) *Ovarian follicles, stimulated by ovulation medications, visible on ultrasound.*

(86,87)

5. Oocyte Retrieval: The follicles were punctured 36 hours after hCG administration. Using transvaginal ultrasound directed approach.

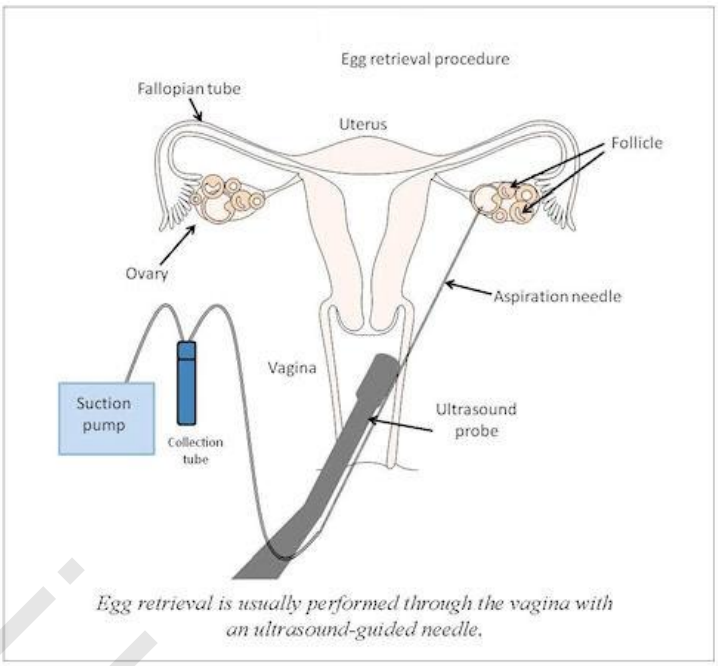


Figure (5): Egg retriveal through the vagina with an ultrasound guided needle.(88)

6. Sperm selection: Selection of the spermatozoon and immobilization. Selection of normal spermatozoon was accomplished by observing its shape, its slight refraction, and its motion pattern in viscous medium. Gentle immobilization achieved through mechanical pressure is needed to permeabilize the membrane that allow the release of a sperm cytosolic factor resulting in oocyte activation and improved fertilization rates.
7. Intra-cytoplasmic sperm injection:

A single, living, immobilized spermatozoon was aspirated tail first into the injecting pipette. The oocyte after removal of the cumulus and corona cells was fixed on the holding pipette in a way that the polar body was situated at 12 o'clock while the injection pipette was pushed through the zona-pellucida at the 3 o'clock position and into the cytoplasm, where the sperm was delivered together with the smallest possible amount of medium.

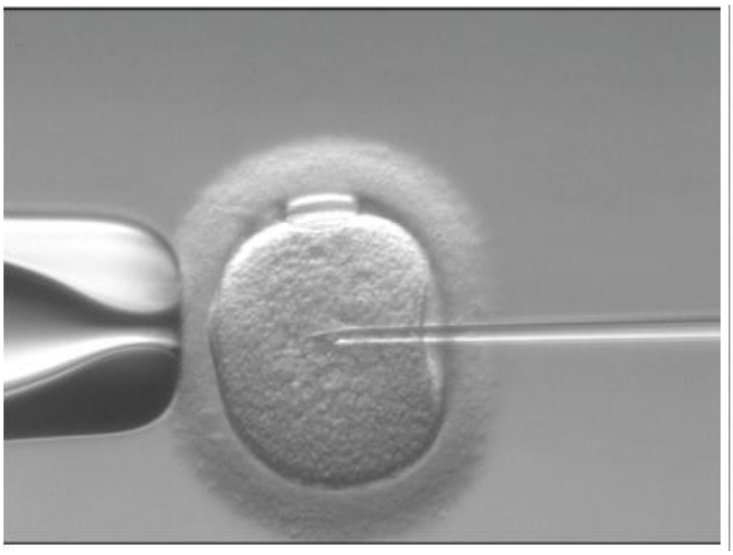


Figure (6): Intracytoplasmic sperm injection.[89]

8. Fertilization was examined 16-18 h after injection by identifying two polar bodies, two pronucleoli and a fine granulated cytoplasm, with dark ring in the middle and a clear halo around the edges. The time for the assessment procedure should be kept as short as possible. and embryo development was evaluated on day 2 and 3 after injection.
9. The proposed embryo score is based on five criteria;
   a) The number of blastomeres or cells observed in relation to number of hours post-ICSI,
   b) The uniformity of cells in terms of size
   c) Cells shape,
   d) The clarity of the cytoplasm in terms of presence or absence of granulation,
   e) As well as the degree of anuclear fragmentation. The best embryos obtained a score of five, while the minimum cut-off score for embryos deemed suitable for transfer was three. (90)

Table I. The five-point embryo scoring system

Features of the embryo	Yes	No
Is the embryo at a 4-cell stage at 44 hr, or a 6–8 cell stage at 68 hr, post-insemination?	1	0
Are all cells uniform in size?	1	0
Are all cells uniform in shape?	1	0
Is the cytoplasm of cells clear?	1	0
Are the anuclear fragments absent?	1	0
If present, do they exceed 25%?	-1	0

10. Three-five embryos were transferred according to the patient's age and embryo scoring. Generally, embryos were transferred to the uterus on the third day after insemination, by which time they have usually divided into six to eight cells.
    Embryos were transferred together in a tiny drop of culture medium using a soft plastic embryo transfer catheters (Labotect). Transabdominal ultrasonography facilitated the transfer procedure because the full bladder needed for an ultrasound scan tends to reduce anteversion of the uterus. It is also reassuring to patient and clinician to see the transfer catheter placed correctly in the endometrial cavity. The procedure was painless and the patient was discharged shortly after transfer. Embryos of good morphological grade in excess of those transferred were cryopreserved for future use.
11. Luteal support: Although in natural cycles the ovary produces progesterone after ovulation, there is evidence of premature luteolysis in some superovulatory regimens. In this study daily intramuscular injection of 100 mg Prontogest® (IBSA) was given.
12. Pregnancy was confirmed by serum β-HCG measurement 16 days after embryos transfer and clinical pregnancy was defined as the presence of a gestational sac on ultrasound scan performed 2 weeks thereafter, with the fetal pulsation.

Statistical Analysis

The obtained clinical data were statistically analyzed using the Predictive Analytics Software (PASW Statistics 18).

1. **Qualitative data:** were described using number and percent. Association between categorical variables was tested using **Chi-square test.** When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using **Monte Carlo correction**.
2. **Quantitative data:** were described using median, minimum and maximum as well as mean and standard deviation.

The distributions of quantitative variables were tested for normality using **Kolmogorov-Smirnov test and Shapiro-Wilk test.**

D'Agstino test was used if there was a conflict between the two previous tests. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used.

- **For normally distributed data:** comparison between more than two population using **Analyzed F-test (ANOVA) and Post Hoc test (LSD).**
- **For abnormally distributed data: Mann-Whitney Test** (for data distribution that was significantly deviated from normal) were used to analyze two independent population. If more than two population were analyzed **Kruskal Wallis** test to be used.

Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

RESULTS

This study was performed in El shatby maternity University Hospital on one hundred patients scheduled for intracytoplasmic sperm injection. All cases were selected after fulfilling criteria of inclusion into the study. A cumulative scoring system was successfully performed with no technical problems.

I) Descriptive data :

1- Age (in years):

Table II: Patients groups according to age.

Age	Patients (%)
Less than 36 years	62%
36-38 years	26%
>38 – <40 years	12%

The mean age for all patients was 35.3 years (standard deviation 5.2 years). 62% of patients were less than 36 years, 26% were between 36 and 38, while 12% were in the 38-40 years age group.

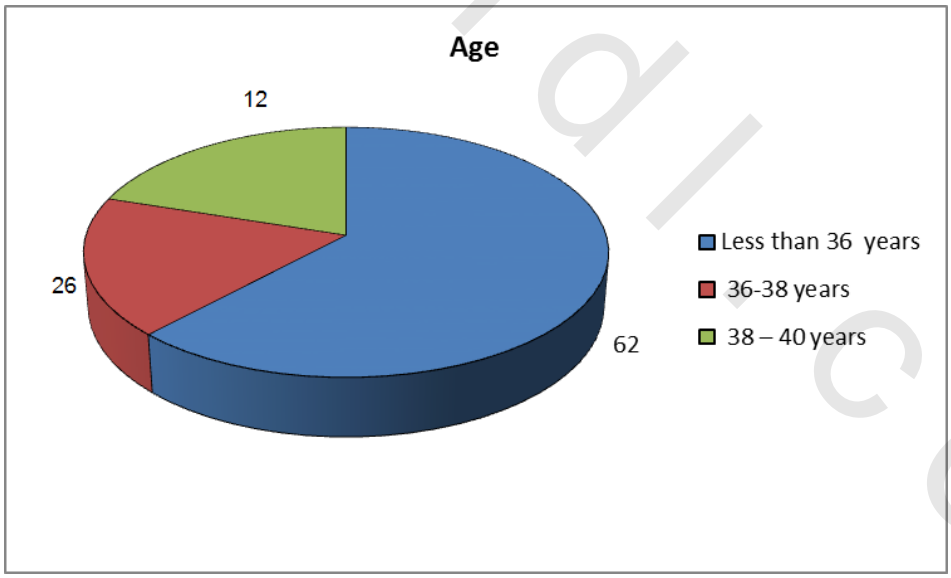


Figure (7): Patients groups according to the age.

Table III: Relation between age & clinical pregnancy rate.

Age	Total No.	Pregnant	Clinical pregnancy rate (%)
Less than 36	62	31	50 %
36-38	26	11	42.3 %
38-40	12	4	33.3 %
Total	100	46	46.0%
X^2	16.25		
P	0.001*		

We found that with increasing age there was decrease in clinical pregnancy rate. However, we did not find a significant difference in age distribution when we compared the pregnant and non-pregnant patients.

2- Body mass index (BMI):

BMI ranged between 27-34 and 26-33 with the mean of 31.05 +/- 2.104 and 30.28 +/- 2.052 for non-pregnant and pregnant groups respectively.

Table IV: BMI in relation to outcome.

	N	Min.	Max.	Mean	S.D.	t-test	p
Non Pregnant	54	27	34	31.05	2.104	1.347	.253
Pregnant	46	26	33	30.28	2.052		
Total	100	26	34	30.70	2.090		

There were no statistical significant differences between pregnant and non-pregnant groups according to BMI. (P=0.253).

3- Etiology of infertility:

Table V: Different causes of infertility and thier percentages.

Cause of Infertility	No. of Patients	%
Male factor	41	41
Tubal factor	31	31
Multiple factors	13	13
Unexplained infertility	9	9
Endometriosis	3	3
Others	3	3
Total	100	100

Male factor and Tubal factor accounted for the majority of cases in this patient set.

4- Duration of infertility:

Table VI: Patients groups according to duration of infertility.

Duration of infertility	Patients (%)
Less than 2 years	42%
2-5 years	46%
> 5 years	12%

The mean duration of infertility for all patients was 3 years (standard deviation 0.2 years). 42% of patients with infertilty less than 2 years , 46% were between 2 and 5 years, while 12% have infertility for more than 5 years.

Table VII: Relation between the duration of infertility and pregnancy rate.

Duration of	Pregnant	Non pregnant	Total

infertility	No.	%	No.	%	No.	%
< 2 years	19	45.2	23	54.8	42	42
2-5 years	22	47.8	24	52.2	46	46
> 5 years	5	41.7	7	58.3	12	12
Total	46	46.0	54	54.0	100	100
p	0.352					

5- Number of embryos transferred:

Table VIII: Relationship between number of embryos transferred and age:

Age	Number of patients	Mean number of embryos transferred (±SD)
Less than 36 years	62	3.3 ± 0.65
36-38 years	26	3.5 ± 0.97
38 – 40 years	12	4.3 ± 1.45
F P	2.06 0.097	

The number of embryos transferred to patients was based on the number and quality of embryos available and the age of patient.

Total number of embryos transferred was 371 embryos from 100 patients

II) Comparative analysis according to day 3 Cumulative Embryo Score items:

1- Blastomere number:

Table IX: Relationship between blastomere number at day 3, number of embryos, number of patients and clinical pregnancy rate.

Blastomere number	No. of embryos	No. of patients	Clinical pregnancy rate
8 or more	212	54	61.3%
Below 8	159	46	28.9%
p	0.369		

Where the average blastomere number of all embryo transferred was 8 or higher the clinical pregnancy rate was more than 60%

In contrast if the average blastomere no. in embryos transferred fell below 8 the clinical pregnancy rate was less than 30 %

There was no statistical significant difference.

2- Blastomere size form:

Table X: Relationship between blastomere size, number of embryos, number of patients and clinical pregnancy rate.

Blastomere size	No. of embryos	No. of patients	Clinical pregnancy rate
uniform	253	64	53%
Uneven	112	36	34%
p	0.0366*		

the clinical pregnancy rate was more than 53% in uniform cell sized embryos, but decrease to 34% in uneven cell size.

There was no statistical significant difference.

3- Blastomere regularity:

Table XI: Relationship between blastomere shape, number of embryos, number of patients and clinical pregnancy rate.

Blastomere shape	No. of embryos	No. of patients	Clinical pregnancy rate
Regular	198	56	46%
Irregular	173	44	29%
p	0.031*		

the clinical pregnancy rate was more than 46% with regular shape blastomere, but fell below 29% with irregular shape blastomere.

There was no statistical significant difference.

4- Blastomere cytoplasm:[91]

Table XII: Relationship between blastomere cytoplasm, number of embryos, number of patients and clinical pregnancy rate.

Blastomere cytoplasm	No. of embryos	No. of patients	Clinical pregnancy rate
None or one pitted	174	48	46.5%
Two or more pitted	197	52	41.3%
P	0.743		

No SD was observed in cinlical pregnancy rates with the transfer of one or more pitted embryos, yet the pregnancy rates did vary for different numbers of pitted embryos transferred.

5- Anuclear fragments: [92]

Table XIII: Relationship between anuclear fragments, number of embryos, number of patients and clinical pregnancy rate.

Anuclear fragments	No. of embryos	No. of patients	Clinical pregnancy rate
No fragments	166	43	25.9%
Fragments	205	57	27.8%
P	0.652		

In absence of anuclear fragments the clinical pregnancy rates reach 68% of transferred embryo. With nuclear fragments pregnancy rates fell below 42%

There was no statistical significant difference.

 # Cumulative Embryo Score:

Table XIV: Five points cumulative embryo scoring system at day 3.

Points	Yes	No
Number of blastomere 8 or more	1	0
Blastomere size uniformity	1	0
Blastomere shape regularity	1	0
Clear cytoplasm	1	0
Absent anuclear fragments	1	0

The best embryos obtained a score of five, while the minimum cut-off score for embryos deemed suitable for transfer was three.

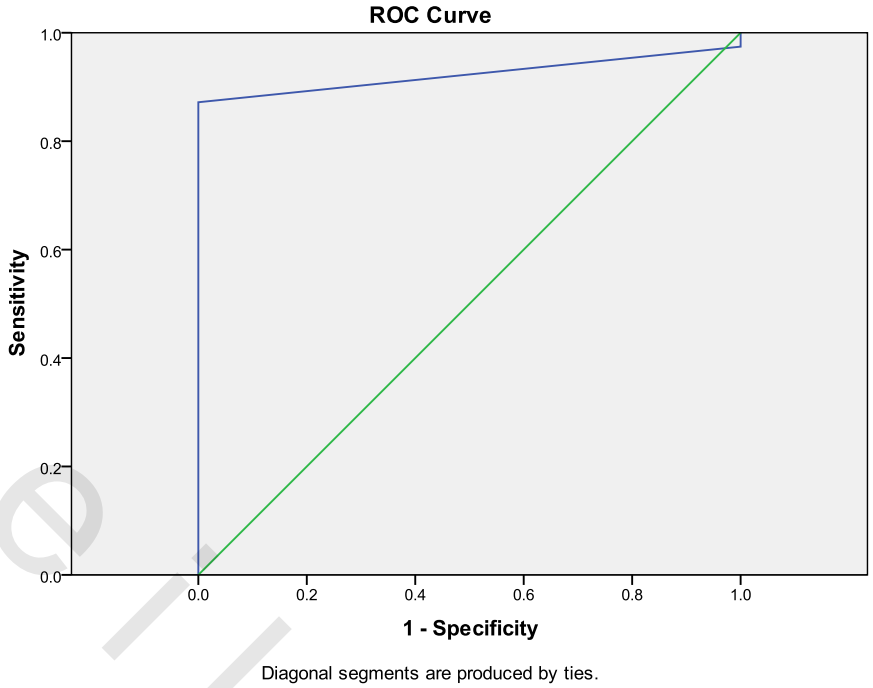
Table XV: Relation between the pregnancy rate and the embryo score.

	Minimum	Maximum	Mean	Std. Deviation
Non Pregnant	2.00	4.00	3.27	0.90
Pregnant	3.00	5.00	4.041	.780
Total	2.00	5.00	4.1000	.904
t-test	71.35			
p	0.0001*			

The mean score of non-pregnant patients was 3, the minimum score was 2, while the maximum score was 4.

The mean score of pregnant patients was 4, the minimum score was 3, while the maximum score was 5.

ROC curve to determine the cut off value of score and its sensitivity and specificity in predicting the pregnant cases.



The scoring system showed sensitivity of 87% and specificity of 100% in classifying transfer cycles into pregnant and non-pregnant with a cut-off point at 3.5 (P <0.05)

Area Under the Curve

Test Result Variable(s):Score 1.

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.923	.027	.000	.870	.976

The test result variable(s): Score 1 has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption.
b. Null hypothesis: true area = 0.5

Coordinates of the Curve

Test Result Variable(s):Score 1

Cut off value	Sensitivity	Specificity
3.5000	.872	100.0