OOCYTE SCORE AND PREGNANCY RATE IN INTRACYTOPLASMIC SPERM INJECTION

By

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ABSTRACT:

Aim of the Work: evaluation of pregnancy rate according to oocyte score and the resulting embryo grades in ICSI cycles in patients receiving long controlled ovarian hyperstimulation protocol. **Patients and Methods:** prospective study included a population of 100 patients complaining of female infertility and aiming to do ICSI. Evaluate the Oocyte score and pregnancy rate after ovulation induction by long controlled ovarian hyperstimulation protocol. **Result(s):** oocytes with higher score recorded high-significant difference ($p<0.001$) between positive and negative pregnancy in all of: polar body, cytoplasm appearance, zonapellucida, granuloza cells, coronal cells, total score ≤5 and number of oocyte. The highest clinical pregnancy rate was derived from the best grade embryos. **Conclusion(s):** morphological assessment of oocytes plays an important role in ICSI outcome, related with that the best grade embryos and the highest clinical pregnancy rate was derived from oocytes with higher score, suggesting that these oocytes were the most synchronous group with respect to nuclear and cytoplasmic maturation.

Key words: oocyte score, ICSI, pregnancy outcome.

INTRODUCTION:

Infertility affects up to 10 -15 % of couples all over the world **(Royal College of Obstetricians and Gynecologists, 2004)**; a proportion of these couples may be able to ultimately conceive. However, for the majority; conception is unlikely without some form of medical intervention **(Collin, 2004)**. Accurate assessment of oocyte maturity in ICSI patient is critical to the timing and success of insemination, whereas errors in assessment may lead to abnormal fertilization and/or poor development potential **(Sathananthan and Trounson, 2006).** A rapid and accurate assessment of oocyte maturity at retrieval and a pre-embryo grading system are important components of any ART program. A standardized, universal scoring system may facilitate communication among embryologists, serve as an adjunct in patient counseling, and most importantly, improve the quality and outcome of ART procedures **(Staessen et al., 1992).** This study aimed to evaluate pregnancy rate and embryo quality according to oocyte score in ICSI cycles in patients receiving long controlled ovarian hyperstimulation protocol.

PATIENTS AND METHODS:

This a prospective study designed in the International Islamic Center for Population Studies and Research from October 2015 to July 2016 to evaluate the oocyte score and embryo quality and pregnancy rate after ovulation induction by long controlled ovarian hyperstimulation protocol 100femalepatients were chosen , all were aged between 25-35 years, body mass index between 18-25 kg/m2, free of medical disorders, first ICSI trial with normal male factor. Basal hormonal profile was performed and all received the long protocol of controlled ovarian hyperstimulation. Induction of ovulation was given when at least four dominant follicles more than 16mm was found on both ovaries.

Oocyte retrieval was performed 34-36 hours from Human Chorionic Gonadotrophin injection (10,000 IU) and oocyte was denudated then checked for maturity and given a score according to **Hill et al., 1989 (Table1& Fig 1&2),** and by **Mohammad et al., 2005** and **Laura et al., 2012** :the morphological feature of each

oocyte was evaluated with the aid of inverted microscope(Olympus 1x71). The criteria employed for the morphological evaluation of oocytes were: a. normal oocytes showed clear cytoplasm with homogenous fine granularity; b. granular oocytes, dark with granularity either homogenous in whole cytoplasm or concentrated in the central portion of oocyte; c. cytoplasmic inclusions comprised vacuoles presumed to be of endocytotic origin; d. anomalies of ZP, e. fragmented polar body of different sizes; f. non-spherical shape of oocyte; and g. wide PVS.

Table(1): the oocytes were assessed oocyte grading (Hill et al 1989)

Grade	Characteristics
Grade 1 (immature oocyte, GV)	Shows a centrally located germinal vesicle. No polar body present.
Grade 2 (nearly mature, metahase I)	No polar body, no germinal vesicle.
Grade 3 (mature/ preovulatory, metphase II)	Sometimes appearing loosely aggregated extruded polar body, no nucleus. Clear ooplasm, homogeneously granulated
Grade 4 (postmature)	Polar body is still intact or fragmented Ooplasm may be slightly darkened, mainly granulated. Oocyte is still round.
Grade 5 (atretic nonviable)	Atresia oocurs in all oocytes from early immature to postmature stages Polar body and nucleus are degenerated, if present. Ooplasm is dark and vacuolated. Uneven surface and very irregular shape of the oocyte; a preivitelline space is obvious clearly visible dark (brush-like) zonapellucida.

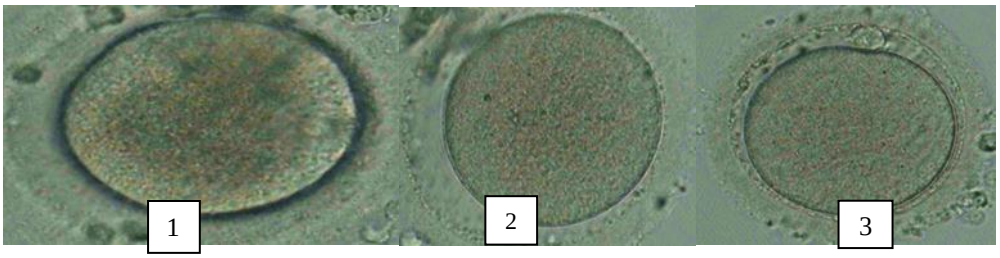

Fig. (1) : photomicrographs of oocyte at germinal vesicle stage (GV) A , oocyte at metaphase I stage B and oocyte at metaphase II stage C (X 200).

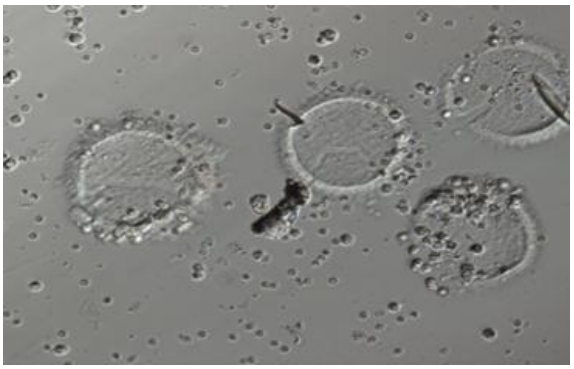

Fig. (2): photomicrographs of empty follicle stages (X 200)**.**

Then all metaphase two (MII) oocyte were subjected to ICSI, 48 h later fertilization was checked and embryo quality was assessed, embryos then transferred and hormonal support by progesterone 100 mg ampules was given, 14 days later chemical pregnancy test is performed.

The ICSI procedure involves the injection of a single motile spermatozoon into the oocyte. The procedure was carried out in a falcon dish containing micro-droplets of cleavage cook culture media and droplet of polyvinylpyrrolidone (PVP) media in the center for sperm immoblization covered with mineral oil **(David, 2007).**

RESULTS:

Table (1): comparison between positive and negative pregnancy cases according to oocyte score and total number of oocyte retrieved (according to Laura et al., 2012).

	Positive pregnancy		Negative pregnancy		P. value
	No.	%	No.	%	
Polar body					
score1(low score)	5	13.5	24	35.3	0.001**
score2(high score)	32	86.5	44	64.7	0.169
Cytoplasm					
score1(low score)	15	40.5	32	47.1	0.013*
score2(high score)	22	59.5	36	59.2	0.066
Zona pellucida					
score1(low score)	5	13.5	48	70.6	0.001**
score2(high score)	32	86.5	20	29.4	0.096
Granuloza Cells					
score1(low score)	2	5.4	38	55.9	0.001**
score2(high score)	35	94.6	30	44.1	0.535
Coronal Cells					
score1(low score)	6	16.2	43	63.2	0.001**
score2(high score)	31	83.8	25	36.8	0.423
Total score					
Total score<5	0	0.0	18	26.5	0.001**
Total score>5	37	100.0	50	73.5	0.163
Number of oocyte					
No of oocyte <6	8	21.6	40	58.8	<0.001**
No of oocyte >6	29	78.4	28	41.4	0.895

The data from table 1 recorded that, there were high-significant difference ($p<0.001$) between positive and negative pregnancy in all of: polar body, cytoplasm appearance, zona pellucida, granuloza cells, coronal cells, total score ≤5 and number of oocyte. While score2 (high score) showed no significance difference.

Table (2): comparison between positive and negative pregnancy cases according to number of grade A embryos presented.

Number of grade A embryos presented	Positive pregnancy		Negative pregnancy		P. value
	No.	%	No.	%	
0	0	0.0	61	89.7	<0.001**
1	0	0.0	7	10.3	
3	5	13.5	0	0.0	
4	9	24.3	0	0.0	
5	6	16.2	0	0.0	
6	7	18.9	0	0.0	
7	4	10.8	0	0.0	
8	2	5.4	0	0.0	
9	1	2.7	0	0.0	
10	3	8.1	0	0.0	

This table illustrated a highly significant ($p<0.001$) increase in the number of grade A embryos presented in positive pregnancy than negative test.

Table (3): comparison between positive and negative pregnancy cases according to number of grade B embryos presented.

Number of grade B embryos presented	Positive pregnancy		Negative pregnancy		P. value
	No.	%	No.	%	
0	1	2.7	13	19.1	0.121
1	15	40.5	26	38.2	
2	17	45.9	20	29.4	
3	4	10.8	8	11.8	
4	0	0.0	1	1.5	

This table recorded increased in grade B embryos presented in negative pregnancy group than positive test, but these showed no significant difference (p = 0.121).

Table (4): comparison between positive and negative cases according to number of grade C embryos presented

Number of grade C embryos presented	Positive pregnancy		Negative pregnancy		P. value
	No.	%	No.	%	
0	37	100.0	8	11.8	<0.001**
1	0	0.0	28	41.2	
2	0	0.0	30	44.1	
3	0	0.0	2	2.9	

This table showed the numbers of grade C embryos presented as the following: there were no case having embryos grade C in positive pregnancy test, while in negative pregnancy test the number of grade C embryos recorded statistically significant (P <0.001).

Table 5: comparison between positive and negative pregnancy cases according to the total number of fertilized oocytes and embryo grading.

	Positive pregnancy		Negative pregnancy		P. value
	No.	%	No.	%	
Total fertilization	267	57.8	195	42.2	**<0.001****
Grade A	206	77.2	7	3.6	
Grade B	61	22.8	94	48.2	
Grade C	0	0.0	94	48.2	

This table showed an increase in the total number of fertilized oocyte and grade A embryos and decreased in grade B and C between positive and negative pregnancy, the difference was statistically significant (P v<0.001).

Table (6): correlation between number of oocytes, total score, numbers of fertilized oocytes and embryo grade.

	Positive pregnancy		**Negative pregnancy**		**P. value**
	No.	**%**	**No.**	**%**	
Total fertilization	267	57.8	195	42.2	<0.001**
Grade A	206	77.2	7	3.6	
Grade B	61	22.8	94	48.2	
Grade C	0	0.0	94	48.2	

The data recorded a strong positive relation between increasing the number of oocytes, number of fertilized oocytes and grade A and grade B, and the decrease grade C embryos with the increasing in the total oocytes score and the difference was statistically highly significant for all.

DISCUSSION:

Intracytoplasmic sperm injection (ICSI) cycles are highly complex procedures, before clinical pregnancy and live birth could be achieved; several critical steps are required including assessment of oocyte morphology.

Non-invasive selection of developmentally competent human oocytes may increase the overall efficiency of human assisted reproduction. The purpose of this study was to summarize the oocyte, embryo quality and pregnancy rate of morphological features of the oocyte that could be obtained by inverted or stereo microscopic investigations.

In the case of ICSI, a rapid evaluation using an inverted microscope is performed after denudation, including evaluation of the oocyte cytoplasm, the polar body, the zona pellucida, the granulosa cells and the coronal cells. This evaluation provides approximate information about the stage of development [germinal vesicle, metaphase I (MI) or metaphase II (MII) phase] and the quality [degenerative signs in the cytoplasm or zonapellucida]. Subsequently all MII oocytes are subjected to ICSI,

and from that point the developmental potential of the obtained embryo is estimated exclusively on the basis of the morphology of the embryo proper, regardless of the quality of the oocyte it was derived from **Laura, et al. (2012)**.

In our study, a correlation was founded between the appearance of the cytoplasm and the oocyte score, fertilization rate, embryo grade and clinical pregnancy rate, These agreed with **Ten et al., (2007)**, **Esfandiari et al., (2006), Rienzi et al., (2008) and Balaban** and **Verman, (2008)** in 'dark cytoplasm–granular cytoplasm' and diffused cytoplasmic granularity'. In another study, homogenous granulation was not mentioned, but two groups of heterogenous granulations were distinguished: on one or two sides of the oocyte, or in the centre **(Wilding et al., 2007).** Compromised quality of embryos which developed from oocytes with dark cytoplasm was reported by **Ten et al. (2007.)**

Diffuse peripheral granulation found to be associated with compromised pronuclear morphology (Rienzi et al., 2008). According to (Wilding et al., 2007), however, any type of cytoplasmic granulation was associated with higher fertilization rates than in oocytes with total absence of granularity. These were in harmony with our results.

Although fertilization rates and embryo quality were not affected (Ten et al., 2007) the presence of vacuoles in oocytes was negatively correlated with cryosurvival and developmental competence of embryos after fertilization (Balaban et al., 2008). Increased biochemical pregnancy rates were followed by decreased clinical pregnancy rates after transfer of embryos derived from oocytes with vacuoles **(Otsuki et al., 2004)**. According to some investigators, cytoplasmic inclusions did not appear to affect fertilization, embryo quality and implantation rates. Others, however, reported decreased fertilization and embryo development **(Otsuki et al., 2007).** According to **(Wilding et al., 2007)** these oocytes also had lower fertilization, embryo developmental and higher aneuploidy rates. When analysed separately the predictive role of presence of vacuoles, smooth endoplasmic reticulum clusters and refractive bodies, **(Rienzi et al., 2008)** have only found a slight but significant decrease in fertilization rates of vacuolated oocytes.

The prognostic value of 1st PB morphology for embryo quality obtained after ICSI was assessed in this study and a correlation was founded between the 1st PB morphology and the oocyte score, fertilization rate, embryo grade and clinical pregnancy rate. The prognostic value of 1st PB morphology for embryo quality obtained after ICSI was assessed alone or in combination with other criteria. Furthermore, it is reported that higher quality embryos are obtained from oocytes having the 1st PB intact and a normal perivitelline space as if to suggest that oocytes with these characteristics are those with the best synchrony between cytoplasmic and nuclear maturation. Furthermore, the encouraging results of **(Ebner et al., 2000)** suggest a significant prognostic role of the 1st PB morphology In another study, poorer results using oocytes having an immature polar body were reported **(Xia et al., 1997)**. It was reported that the ability for complete activation and normal development was achieved by human oocytes during the MII arrest stage. MII oocytes require further time for complete ooplasm maturation in order to be readily activated by the sperm **(Eichenlaub-Ritter et al., 2003; Balakier et al., 2004).**

Better fertilization rate, embryo quality, pregnancy and implantation rate in relation to 1st PB morphology also reported in the previous results, theses agreed with our results. Also in agreement with Ebner et al., 2002in which they found no significant difference between 1st PB morphology and the fertilization rate.

Our results did not agree with **(Verlinsky et al., 2003)** study in which PB morphology is not correlated with the percentage of fertilization, embryo quality, blastocyst survival and outcome.

In this study, a correlation found between appropriate thickness of zona pellucida for developmental stage and the increase in oocyte score, fertilization rate, embryo quality and pregnancy rate. In other studies darkness of the zona did not influence fertilization rates, embryo quality and implantation rates Ten et al., 2007,or cry survival of embryos and subsequent blastocyst and hatching rates (**Balaban and Verman, 2008).**

Our study is in agreement with several studies which found that there was no correlation between the thickness (**EunJeong et al., 2015)** and thickness associated

with darkness **(Rienzi et al., 2008)** on fertilization, pronuclear morphology, embryo development and clinical pregnancy.**(Shen et al., 2005)**have found increased inner layer thickness reported to correlate with increased blastocyst rates, and higher embryo development and clinical pregnancy rates; increased zonapellucida thickness variation was associated with increased embryo quality.

Gaberielsen et al. (2001), state that the thickness of the zona pellucida had no influence on the embryo development after intracytoplasmic sperm injection.
On the other hand, the clinical outcome found improvement when oocytes with high birefringence of the zona pellucida were used **(Shen et al., 2005)**, and low birefringence was correlated with higher miscarriage rates. In one report analysing the effect of multiple morphological features of oocytes on further development, only drastic morphological alterations **(broken or empty zona pellucidae)** were regarded as unsuitable for ICSI.

In this study, we found a correlation between the coronal cells and oocyte score, embryo grade and pregnancy rate and this correlate with **(Ebner et al., 2008)** where he have performed similar grading and had a similar conclusion. In another study Morphological characteristics obtained by non-invasive methods in relation to the further developmental competence were investigated by **(Rattanachaiyanont et al**., **1999)** have graded the expansion of both cumulus and corona radiate individually. According to their results, there was no correlation between the morphology of both cumulus and coronal cells and the fertilization, cleavage and clinical pregnancy rates.

In another study, using a 5-scale scoring system based mostly on the morphology of the cumulus–corona radiate cells; **(Lin et al., 2003)** have found a correlation of the in vitro developmental potential and blastocyst quality. Another scoring system of the quality of the COCs found associations between the observed quality and both fertilization and subsequent pregnancy rates, but not cleavage rates **(Ng et al., 1999).** In an oocyte programme **(Salumets et al. 2002)** have found a strong correlation between the oocyte source and embryo quality whereas cleavage rate was determined by both oocyte and sperm factors.

In this study, we found that embryo grade A is associated with the highest pregnancy rate followed by embryo grade B while embryo grade C associated with no pregnancy rate.Embryo grade A showed high-significant increase with oocyte score 10 than other low scores, Embryo grade B showed high-significant increase with oocyte score 10 than other low scores, Embryo grade C showed significant increase with oocyte score 6 than other high scores.

CONCLUSIONS AND RECOMMENDATIONS:

In conclusion, this study has concluded that the highest clinical pregnancy rate was derived from the best grade embryos that were derived from oocytes with higher oocyte score, suggesting that these oocytes were the most synchronous group with respect to nuclear and cytoplasmic maturation.

As oocyte selection before insemination may have important benefits in saving effort and cost, our finding underlines the importance of more intensive and coordinated research to reach a consensus and exploit fully the predictive potential of morphological examination

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تقييم درجة البويضة ومعدل حدوث الحمل فى الحقن المجهرى

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المقدمة :

يشكل العقم نسبة تصل من ١٠-١٥% من الأزواج في جميع أنحاء العالم ونسبة من هؤلاء الأزواج قد تصبح قادرة على الإنجاب في نهاية المطاف. لكن بالنسبة للأغلبية لا يتصور حدوث حمل بدون اللجوء لمساعدة الإخصاب الطبي المساعد. مؤخرا أصبح الحقن المجهري هو الطريق الأمثل لعلاج أغلبية المشاكل لدى هؤلاء.

وعلى وجه العموم فإن معظم دورات الحقن المجهري تتم مع تنشيط المبيض بهدف إتاحة العديد من الحويصلات والبويضات الناضجة لعملية التخصيب وذلك لزيادة نسبة الإخصاب وزيادة معدلات الحمل.

الهدف من البحث :

يعتبر التقييم الدقيق لنضج البويضة أمر بالغ الأهمية ولنجاح التلقيح وتقييم جودة الأجنة بعد الاخصاب هو أمر بالغ الأهمية في تحديد واختيار عدد الأجنة قبل النقل داخل الرحم (على الرغم من أن عوامل أخرى مثل عمر الأم، وتقبل الرحم تؤثر بلا شك على النتائج). وأيضا فآن المعرفة ألأفضل لخصائص البويضة يؤثر على جودة مرحلة ما قبل الجنين لتقييم هذه الحيوية وارتفاع نسب الحمل الممكنة، والحد من احتمالات المخاطر العالية من تشوهات الأجنة، وتوفير بعض التنبؤ بنجاح الزرع.

الطرق والوسائل العلمية لتطبيق البحث:

وضع تقييم سريع ودقيق لنضج البويضة عند الاخصاب وعمل نظام تدريجي لمرحلة ما قبل الجنين والتي تمكن من بعض التنبؤ بالغرس بعد عملية الحقن المجهري هو من العناصر الهامة في أي برنامج إخصاب مساعد.

إن وضع نظام تسجيل عالمي موحد وقياسي يسهل التواصل بين علماء الأجنة، ويخدم كعامل مساعد في تقديم المشورة للمريض، والأهم من ذلك تحسين نوعية ونتائج الإجراءات في برامج الإخصاب المساعد.

وقد أجريت هذه الدراسة الاستطلاعية في المركز الدولي الإسلامي للدراسات والبحوث السكانية. وكان الهدف من هذه الدراسة الاستطلاعية تقييم معدل الحمل وجودة الأجنة وفقا لمعامل البويضة في حالات الحقن المجهري. وقد تم اختيار١٠٠ مريضة، واللاتي تتراوح أعمارهن بين ٢٥-٣٥ سنة وكان مؤشر كتلة الجسم بين ١٨-٢٥ كجم/م٢ وتم عمل تحليل الهرمونات الأساسية وكان تحليل السائل المنوي لأزواجهن طبيعي، وجميعهن خضعن للبروتوكولات الطويلة من فرط تنشيط المبيض المسيطر. وتم اعطائهن تحريض الإباضة عندما تم العثور على ما لا يقل عن ٤ بويضات مهيمنة أكثر من ١٦مليمتر. وتم سحب البويضات بعدها ب٣٤-٣٦ ساعة ثم تم فحص البويضة لمعرفة مرحلة النضج ومعامل البويضة وفقا للجدول الموضح. ثم خضعت كل بويضة في مرحلة الطور الثاني إلى الحقن المجهري، والتحقق بعد ٤٨ساعة من جودة الأجنة. ثم تم نقل ٢-٣ من الأجنة وإعطاء الدعم الهرموني لمدة ١٤ يوم، ثم تم عمل اختبار الحمل.

نتائج وتوصيات البحث :

تمت جدولة هذه الدراسة ومطابقتها. وخلص إلي أن أعلى معدلات الحمل وأفضل الأجنة قد نشأت من أفضل البويضات درجة. ويوصي بأبحاث مكثفة ومنسقة للتوصل إلى توافق في الآراء والاستفادة الكاملة من الإمكانات التنبؤية للفحص الشكلي للبويضة.