Women Age And Embryo Implantation Following Intracytoplasmic SperminjectionAnd Embryo Transfer In Infertile Patients

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Summary

Background: *The most common cause of reduction in human embryo implantation afterin vitro fertilization and embryo transfer (IVF-ET) is the female age. The increase in the age of women results in reduction in the performance of the reproductive function.*

Objectives: *The aim of the present work was to determine the effect of age of women on intracytoplasmic sperm injection, in vitro embryo cleavage, embryo transfer rate, embryo implantation and pregnancy rates following intracytoplamic sperm injection and embryo transfer (ICSI-ET).*

Patients and Methods: *The male patients had asthenospermia with mean sperm motility of 17.15% and the mean sperm motility index was 31.21. The female patients were divided into three groups, group one, 145 women with age <31 years, group two, 129 women with age group from 31-40 years and group three, 49 women with age >40 years. The ovulation induction was induced by human menopausal gonadotropin (hMG) and human chorionic gonadotropin (hCG).*

Results: *The baseline of FSH and LH levels on cycle day 3 were significantly increased (P<0.05) in group 3 versus group 1 and 2. The estradiol concentration and the number of dominant follicles and thickness of endometrium were significantly decreased in group 3 compared to group 1 and 2 (P<0.01). The number of the hMG ampoules was significantly (P< 0.01) higher in group 3 compared to group 1 and 2. The number of the matured oocytes per patient was significantly lower in group 3 compared to group 1 and 2 (P<0.05). The ICSI rate was significantly higher in group 1 compared to group 2 and 3 (86.01% versus 79.07% and 75.68%, P<0.005). Similar observations were reported in regard to embryo developmental rate and the number of embryo transferred per patient (P<0.05). The differences between group 2 and 3 in regard to the number of embryo transferred per patient were also statistically significant. The percentage of transferable embryo quality was significantly higher in group 1 compared to group 2 and 3 P<0.05) and between group 2 and 3 (P<0.025). The pregnancy rate per patient was significantly reduced in group 3 compared to group 1 (P<0.05). The percentage of fetal sac development per implanted embryo was significantly reduced in group 3 compared to group 1 and 2 (25% versus 66.67%, 64.52%). The percentage of fetal sac development per patient was significantly reduced in group three (>40 years) compared to group one (< 31 years) and group two (31-40 years).*

Conclusions: *It was concluded from the results of the present study that the advancing age of woman (more than 40 years) adversely affects ICSI, embryo transfer , embryo cleavage rates in addition to pregnancy rate. It also reduces embryo implantation and the development of viable gestation sac in the pregnant women. These effects may involve alterations in the function of the endometrium as well as the quality of the oocytes in aged women.*

Key words: *Embryo implantation, ICSI, women age*

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Introduction :

Human embryo implantations following in vitro fertilization and embryo transfer (IVF-ET) are disappointingly low (20-30%) compared to domestic animals such as sheep and cattle (60% success rate). Human embryo implantation is not fully understood.

Despite the laboratory and clinical advancements in ovarian induction, improved in vitro culture conditions and insemination techniques, the IVF-ET outcome remains at low rates in women over 39 years of age [1-3]. The number of oocytes present in the ovary declines with the age of woman due to follicular atresia. This decline in infertility in aged women are not related to diet, stress and sleep. During fetal life, there is a decline in the number of follicles, with an accelerated loss at age of 37-38 years and followed with an increase in serum FSH level [4-5]. Multiple factors affect embryo implantation in IVF-ET. These factors include the etiology, ovarian stimulation protocols, follicular phase estrogen concentrations, the number of egg retrieval, and fertilized oocytes, the quality and

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stage of the transferred embryos, type of luteal support, and the number of the transferred embryos [6-8]. It was concluded from study carried by Hu, et al. [9] that limitation of ET to two good quality embryos in women age 39 or younger eliminate any risk of high-order multiple pregnancies, but that five embryos could be transferred in women over 40 years regardless of morphological quality.

It was found that rapidly divided embryos (fast-developing embryos) are more likely to result in pregnancy and birth of male baby whereas slow-developing embryos showed a reduced developmental rate in vitro and a reduced embryo implantation rate following transfer. It was also observed that pregnancies are usually associated with the transfer of rapidly dividing embryos and grossly delayed embryo development when transferred rarely implanted [10]. The effect of advancing age on IVF-ET outcome is not only manifested in the pattern of ovarian response to gonadotropin action, but also in reduced implantation efficiency [11]. The normal maturation, fertilization and embryonic development of human oocytes are dependent upon a sequence of 3 critical phases
initiated by the ovulatory stimulus. These include 1) a short inductive phases during which the reprogramming of oocyte initiated, 2) a longer "synthetic" phase, which ends shortly after ovulation end is characterized by prominent changes in protein synthesis, and 3) a short and variable postovulatory fertile period during, which the oocyte is receptive to normal fertilization. Irreversible damage occurs if the oocyte is exposed to sperm before the inductive and synthetic phases are completed [12]. Aging of oocytes for a few hours before fertilization reduces the chances of normal embryonic development and associated with embryonic death, chromosomal abnormalities and early implantation failure. The routine culturing of human oocytes for 6-8 hours in vitro after aspiration allow oocytes to complete their nuclear and cytoplasmic maturation developmental lesions, which are induced between aspiration of follicles and implantation of transferred human embryos may impair the implantation rate. These results may provide an explanation for the failure of embryonic growth and low implantation rates in human [13-14]. The infertility decline in aged women may be due to diminished ovarian reserve and it is associated with an increase in spontaneous abortion rates [15].

The objective of the present study was to determine the effect of women's age on embryo implantation and pregnancy rates following ICSI-ET treatment.

Materials And Methods

Patients:

Depending on the age of women, patients were divided in to three groups. Group
one includes 145 females with age less than 31 years. Group two include 129 females with age from 31 to 40 years and group three include 49 females with age more than 40 years. These women had normal ovulatory cycles. The women had completed gynecological investigations including hystrosalpingography or laparoscopy. They were also examined for the presence of antisperm antibodies in their serum and cervical secretions. The concentration of reproductive hormones (FSH, LH, prolactin, estradiol and progesterone) was assayed by radioimmunoassay method and found to be normal in all women involved in this study. The age of the male patients was ranged from 22 to 58 years with mean of 35.34 years. Consultant examined the male patients urologist for presence or absence of varicocele, or other congenital abnormalities.

Complete history regarding sexual habits, venereal diseases or other systemic chronic illness were reported. The major indication for intracytoplasmic sperm injection and embryo transfer (ICSI-ET) was asthenospermia (reduced sperm motility). The male patients had low sperm motility (20% - 30%) and low sperm activity grade (1 – 2) on grade scale from (1-5) and the sperm motility index was also low (40 – 60, normal:180-240) upon examination of semen. Slide agglutination and microagglutination tests (Tray agglutination test) were negative. The duration of infertility was from 6 to 10 years with a mean duration of 7.64 years. Some male patients had mild increase in leukocytic count and received antibiotic treatment for two weeks prior to ICSI treatment.

Ovulation Induction:

The female patient was injected intramuscular injection with 2-3 ampoules of human menopausal gonadotropin (hMG: 75 international unit (IU) FSH, 75 IU LH per ampoule, Pergonal, Serono Company, Italy) per day from cycle day 2 to cycle day 12 or 14 depending on ovarian response. The dose of hMG was monitored by serial measurement of estradiol concentrations. The follicular growth and endometrial thickness was examined by vaginal ultrasound sonography. When the follicular size reached to >16 mm and the estradiol concentration were in the range of 200 – 250 pg/ml per follicle, the female patient received 10,000 IU human chorionic gonadotropin (hCG, Profassi, 5000 IU per ampoule, Serono company, Italy) to induce the final maturation of the follicles. The patients were prepared for oocyte aspiration 36 hours after hCG injection [16].

Semen Analysis:

Seminal fluid analyses were performed to all male patients involved in this study.
The semen was collected by masturbation in clean sterile container. The name, date and time of semen collection was recorded. Sperm concentration, motility, activity grade
(scale from 0-4), normal sperm morphology percentage, sperm agglutination and leukocyte and phagocyte counts were also recorded. Sperm viability was assayed by hypo-osmotic swelling test . Sperm agglutination was examined by slide agglutination and microagglutination tests [16].

Oocyte Rtrieval and Intra-Cytoplasmic Sperm Injection (ICSI):

The oocytes were retrieved under general anesthesia using a vaginal probe transducer (7.0 MHZ, Bruel and Kjaer Co. Denmark), with fixed needle guide. Casmid aspiration needle was used for oocyte aspiration (Casmed 16g, Surrey, UK). A negative pressure of 100-120 mm Hg was applied by suction pump machine (Fugi medical instrument co. Ltd, Japan) and the tip of needle was kept in side the follicle until the follicular fluid was aspirated and resulted in collapse of the follicular wall. The needle was advanced to the next follicle until all the follicles were apirated. Following each aspiration, the needle was withdrawn and flushed by holding the tip of the aspiration needle in a test tube containing flushing culture medium. The procedure was repeated on the other ovary. The follicular fluid was examined under high power stereodissecting microscope (Wild, M3, Heerbrugg, Switzerland) and the maturity of the oocyte was checked. The oocytes were cultured in microdroplet in IVF culture medium (IVF medium, Medicult Co., Denmark) for 4-6 hours before insemination by ICSI.

Denuding micropipette was used to remove the cumulus cells from the oocytes (Billdalsvagen, Billdal, Sweden). The oocyte with the surrounding corona cells and cumulus cells was also transferred to culture medium containing 80 IU/ml hyaluronidase to remove all the cells from the oocytes. Both mechanical and enzymatic procedures were used to clear the oocyte from the surrounding cells. The normality of the oocytes was examined under high power inverted microscope (Nikon inverted microscope, Eclipse TE300, Japan).

The viability of the sperm cells was examined using hypo-osmotic swelling test and only mature morphologically normal oocyte with polar body was used for single sperm injection. The oocyte was held in position by holding pipette (ICSI holding micropipette, ART, CCD Co., France) and the polar body was located at 6 or 12 O'clock position to avoid spindle damage. The injection needle containing immobilized single sperm near the tip of the needle was introduced in the equatorial plane of the oocyte at 3 O'clock position passing through the zona pellucida and oolemma and deposited the sperm in side the cytoplasm. Following the injection of the sperm, the injection pipette was slowly withdrawn. The oocyte was released from the holding pipette and cultured in medium droplet covered with light paraffin oil (liquid paraffin, Medicult Co., Denmark). In next day the fertilization and embryonic development were examined [17-18].

When the embryos reached to 4-cell or 8-cell stage, they were prepared for embryo transfer after >43 hours following insemination. In case of delay embryonic development the embryos were cultured for another 24 hours and transferred later to uterus of the mother using Frydman embryo transfer catheter (Laboratories C.C.D., Paris, France). Human Embryo Transfer The patient was lying in dorsal lithotomic position. The bladder was emptied just Prior to embryo transfer. The cervix was exposed using sterile bivalve speculum and the exocervix was cleaned with sterile culture medium. Frydman catheter was used for embryo transfer. It is a double lumen catheter and has internal and external canals.

The external canal was used for canalization of cervical canal. The internal catheter was used for loading of human embryos and connected to one-ml sterile syringe. The internal catheter after loading of the embryos was passed through the lumen of the external catheter and the embryos were transferred to the uterus.

The internal catheter was washed by culture medium. The Falcon dish containing human embryos was placed on warm plate of microscope stage and the embryos were loaded in side the internal catheter by the following steps: 1) 20 ul was aspirated to inside the catheter 2) followed by 10ul of air, 3) 30 ul of culture medium containing 1-3 embryos was aspirated 4) followed by 10 ul air and 5) finally 20 ul of culture medium. The embryos were loaded between two layers of air bubbles and culture medium in order to avoid surface tension during passage through cervical canal. Pressure was applied on the syringe and the embryos slowly deposited in side the uterus. Following embryo transfer, the internal and external catheters were washed and the wash was examined under microscope to check any retained embryos. The procedure of embryo transfer was repeated in case of retained embryos. The patient was remained in supine position for 20 minutes after embryo transfer and received vaginal progesterone 400 mg/day, Cyclogest suppositories as a luteal support therapy. The B-hCG and progesterone were assayed two weeks after embryo transfer. The embryo implantation was documented by the presence of gestation sac with viable heartbeat following 5 weeks of embryo transfer by ultrasound

examination. The details of ICSI and embryo transfer procedures were described else where [16,19]. The pregnant women were kept on vaginal progesterone therapy for 3-4 months and carefully kept under medical observation (19).

Statistical Analysis:

Data were analyzed using statistical package for social sciences (SPSS 7.5; Inc. Chicago, IL). Data were presented as a mean and standard error of the mean (MSE).

Student t-test, Chi-Square, Bonferroni X square and Kruskal-Wallis one-way analysis of variance was used for analysis of the data.

Results:

The data of semen analyses of group one (<30 years); two (30-40 years) and three (>40 years) are shown in table one. The mean volume of semen in group one was 4.46 ml, in group two was 3.85 and in group three was 4.09 (P>0.05). The sperm concentration (million/ml) was not significantly different between group one, two and three, (39.62, 43.47, 35.86 respectively). The sperm motility and sperm activity grade were similar (P>0.05) in all the studied groups (group one: 18.79%, 1.82, group two: 16.89%, 1.69 and group three: 19.77%, 1.95). The sperm normal morphology and sperm viability was not significantly different among the groups (group one: 45.93%, 66.46%, group two: 48.76%, 62.57% and group three: 43.91%, 64.02%, respectively).

Table 1. Seminal fluid analysis of infertile male patients involved in intracytoplasmic sperm injection and embryo transfer program*

Groups	*Group 1*	*Group 2*	*Group 3*
Patient's No.	*145*	*129*	*49*
Semen volume (ml)	*4.46 ± 2.15*	*3.85 ±3.60*	*4.09 ± 2.09*
Sperm conc. (million/ml)	*39.68 ±8.69*	*43.40 ± 6.94*	*37.52 ±10.73*
Sperm motility (%)	*18.79 ± 2,74*	*16.89 ± 3.85*	*15.77 ± 3.69*
Sperm activity grade	*1.82 ± 0.47*	*1.69 ± 0.37*	*1.95 ± 0.17*
*Sperm viability** (%)*	*66.46 ± 5.27*	*62.39 ± 6.35*	*64.02 ± 6.07*
Sperm normal morph. (%)	*45.68 ± 6.74*	*48. 42 ± 7.31*	*43. 85 ± 5.54*

**Data are mean + SEM, P > 0.05*
***Sperm viability was determined by hypo-osmotic swelling test*

The clinical data of the women involved in intracytoplasmic sperm injection and embryo transfer are shown in table two. The mean age of women in group one was 26.82 years, in group two was 36.78 years and in group three was 42.30. The body mass index (Kg/M2) was not significantly different among the three groups (group one: 24.67, group two: 25.36, and group three: 26.29). The concentration of estradiol (pg/ml) on cycle day three of menstrual cycle was significantly higher (P<0.01) in group one compared to group three (61.82 versus 31.71 respectively). The FSH and LH concentrations in group one were also significantly higher (P<0.05) than that of group three (6.71, 4.23 versus 10.32, 9.56 respectively). The number of stimulation days with human menopausal gonadotropin (hMG) was similar in group one, two and three (10.31, 11.59 and 11.87 respectively, P>0.05). The number of hMG ampoules used for ovulation induction was significantly (P< 0.01) higher in group three compared to group one (31.33 versus 19.37). The number of the dominant follicles and the thickness of endometerium were significantly lower in group three compared to group one (3.12, 6.80 versus 9.88, 9.76 respectively, P< 0.01). The above clinical data of the female patients are shown in table two.

Table 2. The clinical data of the women involved in intracytoplasmic sperm injection and embryo transfer program.

Parameters	*Group one (<31 years)*	*Group two (31-40 years)*	*Group three (>40 years)*
Women's No.	*145*	*129*	*49*
Women's age	*26.82 ± 1.27*	*36.78 ± 2.31*	*42.30 ± 1.85*
Body mass index (Kg/m2)	*23.67 ± 1.36*	*25.36 ± 1.15*	*26.29 ± 2.08*
Estradiol conc. (pg/ml) measured on cycle day 3	*61.82 ± 8.50*	*70.19 ± 4.78*	*31.71 ± 6.89**
FSH conc. (mIU/ml) measured on cycle day 3	*6.71 ± 2.36*	*7.83 ± 1.35*	*10.32 ± 2.86***
LH conc. (mIU/ml) measured on cycle day 3	*4.23 ± 1.34*	*5.51 ± 1.85*	*9.56 ± 2.75***
Days of stimulation with hMG ampoules	*10.31 ±1.22*	*11.59 ± 2.15*	*11.87 ±1.27*
Number of hMG ampoules	*19.37 ± 2.01*	*23.13 ± 1.87*	*31.33 ± 1.26***
Number of dominant follicles > 17 mm	*9.88 ± 1.47*	*7.78 ±1.43 3.12 ± 1.35 **
Endometrial thickness (mm)	*9.76 ± 0.82*	*7.94 ± 0.62*	*6.80 ± 0.71*

**Age group 3 significantly different (P<0.05) from age group 1 and 2.*
***Age group 3 significantly different (P<0.01) from other groups.*

The effect of age of women on ICSI outcome is shown in table three. The number of the retrieved oocytes per patient was significantly lower in age group three compared to age group one and two (3.02 versus 6.40 and 4.66, respectively, (P< 0.05). The percentage of inseminated oocytes was significantly higher (P< 0.05) in age group one compared to age group two and three (86.01% versus 79.07% and 75.68% respectively).

The embryo cleavage percentage was significantly higher in group one compared to group two and three (88.36% versus 78.15% and 67.86% respectively, P< 0.05). The difference in embryo cleavage percentage between group two and three was also significant (P< 0.025). The percentage of transferable quality embryos was significantly higher in group one compared to group two and three (87.54% versus 79.84 and 71.05% respectively, P< 0.5). The number of the embryos transferred per patient was significantly higher in group one compared to group two and three (4.262 versus 2.302 and 1.102 respectively, P< 0.05). The difference between group two and three was also significant in regard to the number of the transferred embryos per patient (P< 0.025).

The pregnancy rate per patient was significantly higher in group one compared to group three (33.10% versus 16.67%, respectively, P < 0.025). The pregnancy rate was not significantly different between group two and three (24.25% versus 16.67%. P > 0.05). The percentage of fetal sac per implanted embryos was significantly lower in group three compared to group one and two (25% versus 66.67% and 64.52%, respectively, P < 0.05). The percentage of viable fetal sac development per patient was significantly reduced in group three compared to group one and two (4.09% versus 22.07%, 15.51%, respectively, P < 0.05, Table 3).

Table 3. The effect of the age of women on intracytoplasmic sperm injection and embryo transfer (ICSI-ET) using semen from asthenospermic men.

Groups	*Group one*	*Group two*	*Group three*	*P values*
	(<31 years)	*(31-40 years)*	*(>40 years)*	
Number of females	*145*	*129*	*49*	*Groups comparisons*
No. oocytes/ patient	*6.4069*	*4.6667*	*3.0204**	**P<0.05*
(No.)	*(929/145)*	*(602/129)*	*(148/49)*	*3 versus (VS) 1 & 2*
ICSI rate (%)	*86.01*	*79.07*	*75.68**	**P<0.05*
	(799/929)	*(476/602)*	*(112/148)*	*1 VS 2 & 3*
Embryo cleavage (%)	*88.36*	*78.16***	*67.86**	**P<0.05: 1 VS 2 & 3*
	(706/799)	*(372/476)*	*(76/112)*	***P<0.025: 2 VS 3*
Transferrable quality	*87.54*	*79.84*	*71.06**	**P<0.05*
embryos (%)	*(618/706)*	*(297/372)*	*(54/76)*	*1 VS 2 & 3*
E.T. per patient	*4.2621*	*2.3024*	*1.1021*	*P<0.05: 1 VS 2 & 3*
	(618/145)	*(297/129)*	*(54/49*	*P<0.025: 2 VS 3*
Pregnancy rate	*33.18*	*24.25*	*16.33**	**P<0.05*
per patient	*(48/145)*	*(31/129)*	*(8/49)*	*3 VS 1*
Fetal sac per	*66.67*	*64.52*	*25**	**P<0.05*
implanted embryos (%)	*(32/48)*	*(20/31)*	*(2/8)*	*3 VS 1 & 2*
Viable fetal sac	*22.07%*	*15.51%*	*4.09%**	**P<0.05*
development/patient			*3 versus 1 & 2*	

Discussion:

Seminal fluid analysis showed that the male patients had marked asthenospermia. Both sperm motility percentages and sperm activity grades were markedly reduced in the studied groups. The concentration of spermatozoa and their normal morphology and viability were normal. Sperm penetration assay (SPA) trials were performed to check the fertilization potential of the sperm using hamster zona-free oocytes and the results showed low penetration score. Intra-Cytoplasmic Sperm Injection (ICSI) was chosen as an alternative to conventional in vitro fertilization (cIVF). Similar technique was applied by other investigator using SPA for IVF or ICSI selection in male patients with low sperm motility (asthenospermia) due to presence of antisperm antibodies [19].

The present study showed that the basal levels of FSH and LH were elevated while the basal level of estradiol was reduced in women aged more than 40 years (group 3) compared to other groups (group 1: <31 years and group 2: 31-40 years). This was followed by a significant increase in the number of hMG ampoules (in women aged > 40 years required for induction of ovulation compared to group 1 and 2) and significant reduction in the number of the dominant follicles and recovered oocytes in addition to significant reduction in endometrial thickness in aged women (>40 years) in the present study. The decreased ovarian reserve and reduction in the effectiveness of the negative feed back mechanism may be responsible for the lower reproductive performance in aged women as compared to younger women [20-21]. The increased hMG ampoules used for ovulation induction in aged group may result in an increase in

serum LH level, which associates with increased follicular fluid androgen, which appears to be detrimental to oocyte development [22-23]. It has been found that premature luteinization or increased follicular androgen production as a result of elevated LH concentration during follicular phase, may impair the normal development of the follicles and results in reduction of oocyte growth and maturation and premature release of progesterone, which lead to premature preparation of endometerium in aged female patients (>40 years old) undergoing ICSI treatment. The significant reduction in ICSI rate, Embryo cleavage rate, embryos of transferable quality rate and the number of the transferred embryos per patients in the age group >40 yeas compared to age group <31 years and age group 31-40 years old women may be due to inferior quality of the oocytes and defect in the preparation of the endometrium for embryo implantation in the more than 40 years old [22, 24]. It has been shown that dominant follicles of older reproductive –aged women contain less granulose cells than that of younger women. The increase in the number of the small follicles and decrease in the number of the dominant follicles in the aged group women of the present study may be responsible for the lower production of inhibin-A that control FSH secretion. It seems that the normal mechanism that restrains FSH secretion in aged women physiologically insufficient compared to younger reproductive-aged women (22).

In the present study the pregnancy rate per embryo transfer and the percentage of viable fetal sac development per patient were significantly decreased in the age group > 40 years compared to age group <31 years in infertile women undergoing ICSI treatment. The effect of advancing age on embryo quality may be the main cause of the reduction in embryo implantation after the age of 40 years. It has been shown by other workers that embryos resulted from oocytes of older women undergoing ICSI treatment have diminished capacity for implantation.

An increase in the maternal age positively correlated with the percentage of chromosomally abnormal embryos 915). The seminal fluid parameters were similar in the present study (P>0.05), therefore the significant reduction in the number and quality of the oocytes as well as the embryo implantation and pregnancy rate are due to the adverse effects of advancing of women age. The present study showed clearly that not all the implanted embryos are capable to develop to normal viable gestation sac. Younger age women (age group < 31 years and age group 31-40 years old) demonstrated significantly higher capacity for fetal sac development compare to women aged more than 40 years (Percentage of viable fetal sac development per patient).

It seems that the endometrial cells have an important function in filtering genetically abnormal embryos and preventing their implantation [25]. The adverse effect of the advancing age of women on the normal development of the endometrium can not be ignored since in the present study endometrium thickness was markedly decreased in aged group (> 40 years) compared to other younger aged groups (<31 and 31-40 years).

In conclusion, the number of the dominant follicles, the concentration of estradiol, the fertilization and the number of the transferable quality embryos as well as pregnant rate per embryo transfer and the development of viable fetal sac following embryo transfer are markedly reduced in more than 40 years aged women. The age of women was found to affect the ovarian reserve as well as the endometrium. The authors suggest transplantation of ICSI embryos derived from >40 years donor women into younger surrogate recipient women.

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References:

1. *Ridha-Brzanji MT, Dukelow WR. The effect of ovarian stimulation hormones transfer media and side of transfer on implantation rates of hamster embryos: model for human embryo transfer. J Facul Med 1990; 32: 39-46.*
2. *Shapiro BS, Harris DC, Richter KS. Predictive value of 72-hours blastomere cell number on blastocyst development and success of subsequent transfer based on the degree of blastocyst development. Fertil Steril 2000; 73: 582-586.*
3. *Schwartz LB, Chiu AS, Courtney M, Krey L, Schmidt-Sarosi C, The embryo versus endometrium controversy revisited at its relates to predicting pregnancy outcome in in-vitro fertilization cycle. Hum Reprod 1997; 12: 45-50.*
4. *Faddy MJ, Gosden RG, Gougeon A. Accelerated disappearance of ovaria follicles in midlife: implantation for forecoasting menopause. Hum Reprod 1992; 7: 1342-1346.*
5. *Leung CKM. Recent advances in clinical aspects of in vitro fertilization. H Kong Med J 2000; 6: 169-176.*
6. *Dukelow WR, Ridha-Brzanji MT. Fertilization and preimplantation embryonic development. In: Brans YW, Kuehl TH (eds.), Nonhuman Primates in Perinatal Research. New York, NY: John Wiley and Sons Inc; 1988: 119-137.*
7 *Steer CV, Mills CI, Tan SL, Campbell S, Edward RG. The cumulative embryo score: a predictive embryo scoring technique to select the optimal number of embryos to transfer in an in-invitro fertilization and embryo transfer program. Hum Reprod 1992; 7: 117-119.*
8 *Gardner DK, Lane M, Stevens, J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: toward a single blastocyst transfer. Fertil Steril 2000; 73: 1155-1158.*

9 Hu Y, Maxson WS, Hoffman DI. Maximizing pregnancy rate and limiting high- order multiple conceptions by determining the optimal number of embryos to transfer based on quality. Fertil Steril 1998; 69: 650-657.

10 Kausche A, Jones M, Trounson AD, Figueiredo MV, Lolatgis, N. Sex ratio and birth weights of infant born as a result of blastocyst transfers compared with early cleavage stage embryo transfers. Fertil Steril 2001; 76: 688-693.

11 Damario MA, Rosenwaks Z. Repeated implantation failure. In: Gardner DK, Weissman A, Howles CM, Shoham Z (eds.), Textbook of Assisted Reproductive Techniques: Laboratory and Clinical Perspectives. London, UK: Martin Dunitz Ltd; 2001: 543-560.

12 Osbora JS, Moor RM. An assessment of the factors causing embryonic loss after fertilization in vitro. J Reprod Fertil 1988; 36: 59-72.

13 Edward RG. Cause of early embryo loss in human pregnancy. Hum Reprod 1986; 1: 185-198.

14 Meldrum DR. Reducing the incidence of multiple gestation. In: gardner DK, Weissman A, Howles CM, Shoham Z (eds.), Textbook of Assisted Reproductive Techniques: Laboratory and Clinical Perspectives. London, UK: Martin Dunitz Ltd; 2001:674-679.

15 Munne S, Alikani M, Tomkin G, Grifo J, Cohen J. Embryo morphology, development rates and maternal age are correlated with choromosomal abnormalities. Fertil Steril 1995; 64: 382-391.

16 Ridha-Brzanji, MT, Al-Anssari, SAM, Balafair, AS, Khunda, SS. Baghdad culture medium and in vitro fertilization of human eggs and embryo transfer. Accepted for publication in journal of Faculty of Medicine, Baghdad 2005.

17. Van de-Velde H, Nagy ZP, Joris H, De-Vos A, Van-Steirteghem AC. Effects of different hyaluronidase concentrations and mechanical procedures for cumulus cell removal on the outcome of intracytoplasmic sperm injection. Hum Reprod 1997; 12: 2246-2250.

18 Ridha-Brzanji MT, Al-Anssari SAM, Khunda SS. Successful life birth following single sperm HOST-ICSI embryo transfer application in TESE patients. Kufa Med J 2002; 5: 20-25.

19 Al-Anssari SAM. The effect of prednisolone and luteal support therapy on implantation potential of human embryos following in vitro fertilization and embryo transfer in immunologically infertile male patients. Ph D Thesis, Baghdad University Medical School 2004: 59-80.

20 Ahmed-Ebbiary NA, Lenton EA, Cooke, I. Hypothalamic pituitary aging :progressive increase in FSH and LH concentrations throughout the reproductive life in regulating menstruating women. Clin Endocrinol 1994; 41: 199-206.

21 Schoolcraft WB, Schlenker T, Jones GS, Jones HW. In vitro fertilization in women age 40 and older: the impact of assisted hatching. J Assist Reprod Genet 1995; 12: 581-584.

22 Hussain SM. Superiority of gonadotropin analogue in ovulation induction and in vitro fertilization and embryo transfer in Iraqi females. Ph D Thesis, Baghdad Univertsity Medical School 2001: 100-112.

23 Jacobs SL, Metzger DA, Dadson WC, Haney AF. Effect of age on response to human menopausal gonadotropin stimulation. J Clin Endocrin Metab 1991; 41: 1525-1530.

24 Janny L, Menezo YJ. Maternal age effect on early human embryonic development and blastocyst formation. Mol Reprod Dev 1996; 45: 31-37.

25 Ridha-Brzanji, MT. Reproductive filters in human body. In: Ridha-Brzanji MT, Al-Adeely SG (eds.), Human IVF and Cloning: Islamic Sight. Beirut, Lebanon: Resalah Publishers Ltd; 2001:79-88.