Comparison Of Pregnancy Outcomes Between Day 2 And Day 3 Embryo Transfer

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Abstract

Objective: To test if the extended embryo culture and embryo selection methods have a positive effect on pregnancy outcome or not.
Materials & Methods: This study had been carried out in infertility unit at C-PLAS hospital at Sana'a, Yemen during the period from July 2007 to July 2009, it includes 305 intracytoplasmic sperm injection cycles had fresh embryo transfer either on day 2, (220 patients) or on day 3, (85 patients). It retrospectively analyzes the pregnancy rates for both groups.

Results: The mean age was similar in both groups. Pregnancy rates were slightly higher in day 3 embryo transfer (43.52 %) versus day 2 embryo transfer (40 %) but not statistically significant. There was no statistical significant difference in pregnancy rates based on the number of embryo transferred in both groups, However there was significant difference in the quality and cleavage stage of embryo, day 2 embryo transfer (grad A 68.14 %, grad B 25.62 % and grade AB 6.23 %, 4 cell 98.19 % and 8 cell 1.8 %) versus day 3 (grad A 85.71 %, grad B 11.84 % and grade AB 2.43 %, 4 cell 49.13 % and 8 cell 50.87 %).

Conclusion: Extending embryo culture period from day 2 to day 3 have positive effect on cleavage stage and quality of embryo, but had no adverse effect on pregnancy rate .Embryo transfer could be done on day 2 or day 3 according to quality and cleavage stage of embryo.

Key word: Embryo quality, cleavage stage, pregnancy rate, day2 and day3 ET

Introduction

Many studies have compare day 2 versus day 3 embryo transfer outcome in intracytoplasmic sperm injection "ICSI, using extended embryo culture together with selection of good quality embryos. Transfer of day 3 embryos should be associated with higher implantation rate and pregnancy rate than transfer of day 2 embryos .Since the start of IVF embryo has been transferred 2 days at 4 cell stage due to lack of suitable culture media able to sustain embryonic development for several days. The timing of arrival of the embryo in the uterus however is premature compared with the situation in vivo where the embryo enters the uterus at morula stage "4-5" days after ovulation (1).

Transfer of embryo to the uterus on day 3 after oocyte retrieval may be closer to physiological time of uterine entry than transfer on day 2. Delaying embryo transfer would allow the selection of the most vital embryos for transfer (2). A retrospective study showed that pregnancy rates were similar between day 2 and day 3 transfers but implantation rate in day 3 groups was higher, (3). The pregnancy and implantation rates were found to be increased after transfer on day 3, (4). Aboulghar et al. 2003, found no significant difference in pregnancy rate between day 2 day 3 embryo transfer (5). As shown in previous studies on day 2 versus day 3 embryos transfer remains controversial. It is useful to perform this study to compare implantation and pregnancy rate between day 2 and day 3 embryo transfers.

Patients and Methods

This study had been carried out in infertility unit at C-PLAS hospital at Sana'a, Yemen during the period from July 2007 to July 2009. It includes 305 patients undergoing infertility treatment by ICSI due to either male or female factor, They were classified into two groups, group one day 2 embryo transfer, group two day 3 embryo transfer.

Both groups were subjected full history taking, general examination, vaginal ultrasound, basal hormonal profile (most normally and some ICSI due to tubal factor and pco) and seminal analysis (All by ejaculate either normal or oligo-athenospermia with normal morphology). All patients were treated using short protocol (as routine for our center in this period) gonadotrophin–releasing hormone "GnRH" decapeptyl 0.1 from first day of menses, then starting human menopausal gonadotrophin "HMG" (150IU TO 300IU) according to age ,weight and response to stimulation and folliculometry follow up then injection of 10000 IU human chorionic gonadotrophin " HCG" was given for oocyte maturation. At 34-36 hours ovum pickup through transvaginal ultrasound guided was prepared.

Embryo culture and embryo transfer procedure Oocytes were identified in the laboratory and briefly rinsed free of follicular fluid and blood in handling Gamete medium (K-SIGB-50; Cook IVF, Australia). Freshly ejaculated semen was washed in Sperm media (K-SISM-50; Cook IVF, Australia) by centrifugation at 1600 rpm for 5min after 30 min liquefaction period. The pellet was further processed by the side migration technique for ICSI as described by (Dozortsev et al., 1996). Oocytes for injection were denuded of cumulus cells following brief exposure to hyaluronidase for >1min (K-SIHY-1-5; Cook IVF, Australia) and then assessed for maturity. MetaphaseII oocytes were injected using the method described by (Dozortsev et al., 1996). Microinjection was carried out on the heated stage of an inverted microscope (Nikon-TE2000-U, Japan). The injected Oocytes were incubated in 50µl drop of fertilization media (K-SIFM-20; Cook IVF, Australia) in culture dish (REF-353004; Falcon, USA) under mineral Oil (K-SICO-200; Cook IVF, Australia) in an incubator containing 6% CO2 in air at 37°C. 16–20 hours post-insemination, oocytes were assessed for fertilization. Those oocytes exhibiting two pronuclei and two polar bodies were placed in one or two groups in <l micro drops of a ready to use Cleavage media (K-SICM-20; Cook IVF, Australia) under mineral oil (K-SICO-200; Cook IVF, Australia) in culture Dish (REF-353004; Falcon, USA). In the day 2 transfer group, embryonic development was assessed under the inverted microscope 42¬¬- 44 hours after ICSI. In the day 3 transfer group, embryos were first evaluated 42-44 hours after ICSI and then for a second time 24 hours after the first evaluation. Embryos were classified based on morphological criteria as described by (Laverge et al., 1997). Briefly, embryos without a nucleated fragments and with equally-sized blastomeres were graded as type I. Embryos with some a nucleated fragments (>10%) and/or with unequally-sized blastomeres were graded as type I-II. Embryos with unequally-sized blastomeres with either =20%, up to 50% or >50% a nucleated fragments were classified as type II, II-III, and III respectively. Embryos of grade I and I-II were classified as excellent quality embryos, embryos of grade II as god quality embryos and embryos of Grade II-III and III as moderate to poor quality embryos. The number for transfer being determine by the availability of embryos for transfer and the patient's age and previous clinical history. If the patient aged 35 years or had failed determine by the availability of embryos for transfer and the patient's age and previous clinical history. If the patient aged 35 years or had failed to achieve a pregnancy after three or more previous IVF cycles then consideration was given to transferring three or four Embryos, if Available, rather than only two embryos, which would be the usual recommendation to patients of a younger age or with a limited IVF history. One to four Embryos were transferred to each patient. Supernumerary embryos up to type II were cryopreserved with a dimethyl sulphoxide (DMS) rapid-freezing protocol. Pregnancy was defined as positive if the ßHCG measured in venous blood was >20 mIU/ml. Clinical pregnancy was defined as a positive pregnancy test followed by the presence of a fetal sac on transvaginal ultrasound 4 weeks after transfer.

Statistical analysis

Data were analyzed using the Minitab software (version 12.1, Minitab Inc.).The Paired t test was used to compare the mean differences in number of embryos transferred in both day 2 and day 3. The Chi-square test was used to compare Pregnancy rate after day 2 and day 3 embryo transfers and quality of embryos transferred. A P value less than 0.05 were considered significant.

Results

The mean age was similar in both groups. Pregnancy rates were slightly higher in day 3 embryo transfer (43.52 %) versus day 2 embryo transfer (40 %) but not statistically significant (Table-2).

There was no statistical significant difference in pregnancy rates based on the number of embryo transferred in both groups (Table-3, 4), however there was significantly difference in the quality and cleavage stage of embryo, day 2 embryo transfer (grad A 68.14 %, grad B 25.62 % and grade AB 6.23 %, 4 cell 98.19 % and 8 cell 1.8 %) versus day 3 (grad A 85.71%, grad B 11.84 % and grade AB 2.43 %, 4 cell 49.13 % and 8 cell 50.87 %) (Table-5).

Table 1:
Show the pregnancy rate in day 2 and day 3 according to patient age.

P value	Pregnancy Rate (%)			Age (Yr)
	Total	Day 3	Day 2	
0.109	26/57 (45.61)	5/17 (29.41)	21/40 (52.05)	18-25
0.336	49/110 (44.54)	17/33 (51.51)	32/77 (41.55)	26-30
0.252	29/77 (37.66)	8/16 (50)	21/61 (34.42)	31-35
0.568	14/35 (40.00)	6/13 (46.15)	8/22 (36.36)	36-39
0.518	7/26 (26.92)	1/6 (16.66)	6/20 (30)	≥ 40

Fig 1:
Show the pregnancy rate in day 2 And day 3 according to patient age

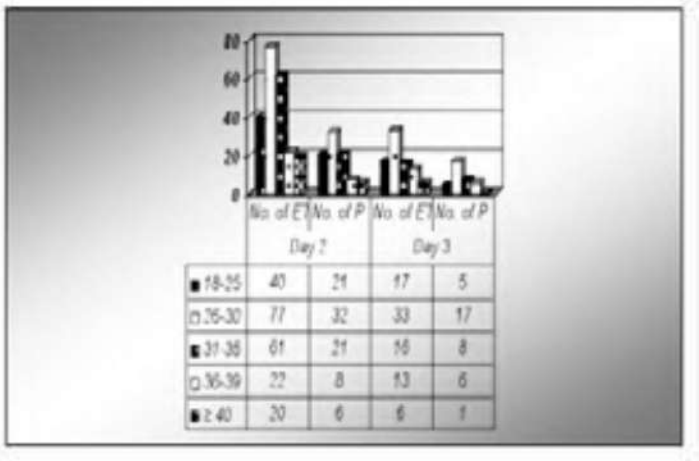


Table 2:
Comparison of pregnancy rate (PR) after Day 2 and Day 3 embryo transfer.

Transfer day	No. of Transfers	No. of Pregnancies	PR (%)	P-value
Day 2	220	88	40.00	0.574
Day 3	85	37	43.52	
Total	305	125	40.98	

Fig 2:
Comparison of pregnancy rate (PR) after Day 2 and day 3 embryo transfer.

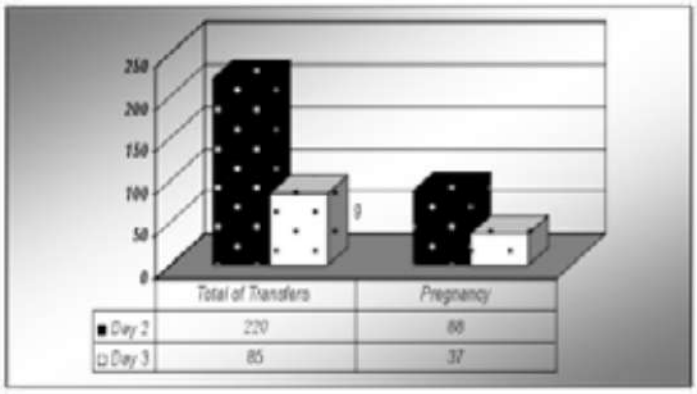


Table 3:
Comparison between the means of the transferred embryos in day 2 and day 3.

Number of Transferred Embryos	Day 2	Day 3	P value
Total No. of ET	722	287	
Mean of Embryos Transferred	3.50±1.32	3.44±1.34	(0.70)

Table 4:
Pregnancy rate (PR) based on the number of embryos transferred (ET).

No. of ET	PR (%)		Total	P value
	Day 2	Day 3		
1-2	16/49 (32.65)	11/22 (50.00)	27/71 (38.02)	0.164
3	12/46 (26.08)	5/12 (41.66)	17/58 (29.31)	0.291
4	34/75 (45.33)	13/34 (38.23)	47/109 (43.11)	0.488
≥5	26/50 (52.00)	8/17 (47.05)	34/67 (50.74)	0.725

Fig 3:
Pregnancy rate (PR) based on the number of embryos transferred (ET).

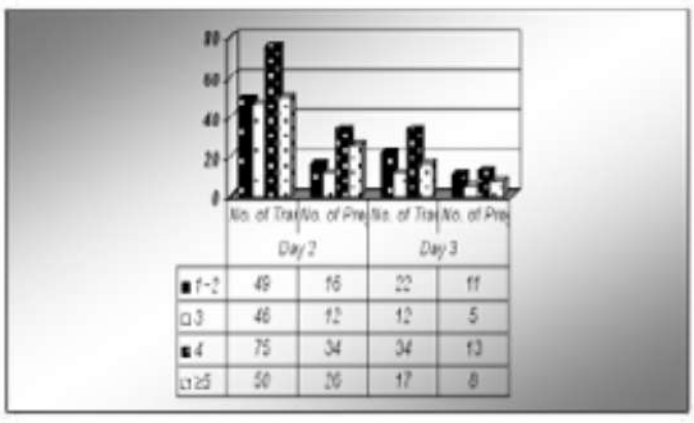
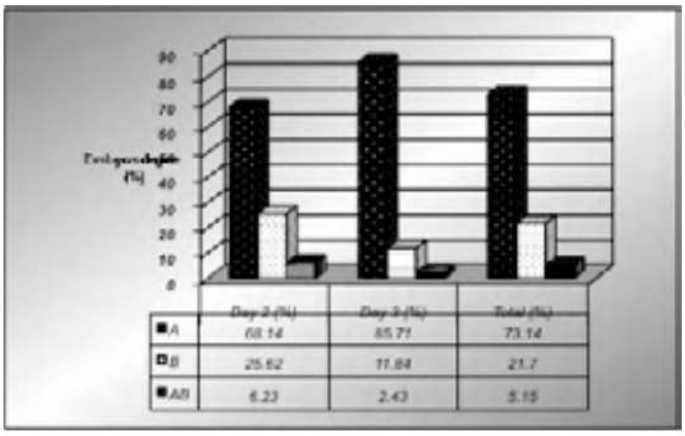


Table 5:
Comparison Embryos quality in day 2 and day 3 based on degree of fragmentation.

Embryo Degree	Day2 (%)	Day3 (%)	Total (%)	P-value
Excellent quality embryos (grade A)	492/722 (68.14)	246/287 (85.71)	738/1009 (73.14)	>0.0001
Good quality embryos (grade B)	185/722 (25.62)	34/287 (11.84)	219/1009 (21.70)	>0.0001
Moderate quality embryo (grade AB)	45/722 (06.23)	7/287 (02.43)	52/1009 (05.15)	0.014
Cleavage stages				
Second stage (>=4 cell)	709/722 (98.19)	141/287 (49.13)	850/1009 (84.24)	>0.0001
Third stage (>=8 cell)	13/722 (01.80)	146/287 (50.87)	159/1009 (15.75)	>0.0001

Fig 4:
Distribution of embryo grades on day 2 and day 3



Discussion

This prospective study compares day 2 and day 3 embryo transfer after oocyte retrieval in 305 patients, who were compared for age and number of embryo transfer but fixed for treatment protocol, all were short protocol. The pregnancy and embryo implantation rates were comparable in day 2 and day 3, although the pregnancy rate was slightly higher after transfer in day 3 than on day 2 (table 2), this difference was not statistically significant. This result is in agreement with results of Edward et al., 1984, Dawson et al., 1995, Oatway et al., 2004 and Ashrafi et al., 2007 (3,6-8).

A number of studies addressing the same issue as Huisman et al., 1994 (9) and Aboulghar et al., 2003 (5) in large retrospective study compared results after day 2 and day 3 embryo transfer, the pregnancy and implantation rates were similar in both groups. However some studies have reported positive effects of embryo transfer on day 3 than day 2 (10,11). Transfer of embryos to the uterus on day 3 after oocyte retrieval may be closer to the physiological time of arrival of embryo to the uterine cavity than transfer on day 2. Moreover delaying embryo transfer would allow the selection of the most vital embryos for transfer (12) and these factors may have

had positive effects. There was no significant difference in number of embryo transfer which was in agreement with (8).

In our study, the selection of excellent quality embryo grade A for transfer is one the most important factors with other factors (as endometrial receptivity, ovarian response and oocyte maturity) for successful ICSI program, this selection based on morphological criteria to select embryo showed better significant difference in day 3 than day 2, these results were in agreement with (13). Also the third cleavage stage was more in day 3 than in day 2. Dawson et al., 1987, reported that there is no difference in embryo quality between day 2 and day 3 in distribution of embryo grads which was in disagreement with our study which may be due to a delay of one day may be too short for use to better differentiate the quality of embryos (3). Delaying embryo transfer until day 3 provides an opportunity to observe the embryos for a further 24 hours in culture. Any morphologically normal embryos on day 2 which subsequently arrest or degenerate can be identified and their transfer avoided .This might have appositive effect on implantation rates and further successful pregnancy outcome.

Some authors believe that some suboptimal quality embryos may be rescued in uterine environment and that extended culture might be a cause of arrest for further development of such embryos (14).

So a large proportion of human embryos will arrest in vitro between the 4 and 8-cell stage (2). The percentage of second cleavage stage on day 2 embryo transfer was (98.19%) as compared to the (50.8%) third cleavage stage on day 3 (table 5). Yet this difference did not improve the pregnancy rate in day 3 over day 2 after we had the opportunity to exclude arrested embryos at 4 and 8 stage (table 5). The human cleavage stage embryo normally resides in the oviduct and does not enter the uterus until after compaction (15). The oviduct and uterus provide different nutrition environment for the embryo (16). In recent years, there for, several investigators (3) have tried amore extended delay of embryo transfer, up to blastocyst stage. Extending the culture period to beyond the time of expected activation of the embryonic genome might optimize the selection of viable embryos for transfer (17).

In addition, by delaying the embryo culture, embryos with limited and any abnormal development potential may be identified and avoided (2). Some chromosomally abnormal embryos fail to develop in culture (18). At present study, the embryo transfer with one day delay not only have no adverse effect on embryo quality and embryo transfer, but also it showed positive effects (non statistically significant) on pregnancy rate. Extending embryo culture period from day 2 to day 3 have positive effect on cleavage stage and quality of embryo, but had no adverse effect on pregnancy rate .Embryo transfer could be done on day 2 or day 3 according to Moreover these finding indicate that embryo transfer can be safely scheduled at the convenience of the patient and the center.

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