RESEARCH
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EFFECTS OF REMOVING FRAGMENTATION AT THE DAY TWO AND THREE OF DEVELOPED BLASTOMERE ON EMBRYO QUALITY IN ICSI CYCLES

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Abstract
Background: During assisted reproductive technologies procedures, fragmentation and cell debris are considered important prognostic factors in the static morphologic assessment of human embryo quality, along with cell number, size, and symmetry. The goal of embryo grading at any stage is to select the embryos with the highest implantation potential to be transferred.
Objective: The present study was aimed to evaluate the effects of removing the fragmentations at day 2 and 3 from developed blastomere on embryo quality of in vitro fertilization/ intra-cytoplasmic sperm injection cycles.
Materials and Methods: This study was considered a retrospective analysis involving 40 couples enrolled and fit for intra cytoplasmic sperm injection (ICSI) program in High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University and Al-Zaitoon Specialized Fertility Center through the period from November 2023 till April 2024. All developed embryos which had over >25% fragmentation (grade 3 or 4) on day 2 and 3 of the - cleavage stage were micromanipulated to eliminate fragmentation. Embryos were divided into two groups depending on the response to cleavage development after removal of fragmentation;1- positive developed embryos group (n=20) and 2- negative embryo non –developed group (n=20). Microsurgical fragment removal was performed by micromanipulation with later-assisted hatching and a handmade suction micropipette that had an outer diameter of 30 µm. All the results of ICSI program was measured and reported.
Results: Insignificant increase of embryos Grade I and II at day 2 after removal of fragmentations with decline in Grade III and Grade IV embryos were observed. There was a significant increase in Grade III embryos (P=0.047) in negative non-developed embryos compared to positive developed embryos after fragmentation removal at day 3.
Conclusions: It is concluded from the results of current study that elimination of fragmentations from cleavage embryos at day 2 and 3 resulted from ICSI program had a positive effect on blastomere quality and embryonic development. Because this study the first Iraqi trail, it is recommended to transfer and/ or cryopreserve these embryos in future to obtain higher percentage of pregnancy rates.
Keyword: blastomere, day2 and 3 embryos development, fragmentation removal, ICSI.

INTRODUCTION

Assisted reproductive technologies (ART) are any fertility-related treatments in which ova or cleavage embryos are manipulated. Assisted reproductive technologies are used to aid in achieving pregnancy conception in individuals who are having difficulty doing through normal physiological status (Vilda et al. 2024). There are numerous techniques for assisted conception includes; intra uterine insemination, in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) and others (Hunter 2024; Taha et al. 2022). Insemination of mature oocytes by mature sperm(s) using any of these procedures leading to normal fertilization (Gardner & Wale 2024). However, through embryonic development there may be different abnormal evidence such as; fragmentation, uneven cell size or number and multinucleation (Li & Gao 2024).

It has been stated that embryos with higher cell numbers, regular cells and little or no fragmentation have a higher chance of implanting than do other embryos with less cells, more irregularity and significant fragmentation (Chavli et al. 2024). Fragmented embryos correlate with poorer prognosis cycles; however, fragmented embryos that undergo defragmentation, result in equivalent clinical outcomes to high-grade of non-defragmented embryos (Zhang et al. 2022; Ahmed et al. 2022). The degree of fragmentation was expressed as a percentage and defined as the volume of the perivitelline space and/or cleavage cavity occupied by anucleate cytoplasmic fragments (Rosen et al. 2019). Based on distribution and size of the fragments (Li & Gao 2024). Therefore, the study aimed to evaluate the effect of elimination the fragmented blastomere on early cleavage embryonic stages following IVF/ ICSI cycle.

PATIENTS, MATERIALS AND METHODS:

The present clinical trial study included 40 infertile women whose age ranging between 23 - 45 years old who attended the infertility consultant clinic and IVF units of High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University and Al-Zaitoon Specialized Fertility Center, Baghdad -Iraq between November 2023 till June 2024. Couples with female factor infertility (e.g. PCOS, poor respond and tubal obstructive), male factor and with unexplained infertility were included in the study. Half of them with primary infertility and the other 50% with secondary infertility. Forty out of 385 developed fertilize oocytes following ICSI procedure were taken on day two and three with different grades of fragmentation.

Ethics committee approval was received for the study, and all patients who participated in this study gave written informed consent forms.

Semen preparation: A complete semen analysis was performed according to the manual of WHO (2021) for sperm concentration, motility, and WHO (1999) for measuring sperm morphology. The men semen was collected in a dry, clean, sterile cup in the day of oocyte retrieval by masturbation, after 2-5 days of abstinence, and then the semen sample was immediately incubated for 30 minutes in a 37°C, to allow to liquefy. If the husband is azoospermic, sperm was surgically harvested from the testes, epididymis, or vas deferens. The method of sperm preparation was done by immediately overlaid the semen sample with sperm washing medium (Vitrolife, Sweden), allowing sperm from the seminal plasma to swim into the medium. Then, the sperm suspension was washed extensively to remove seminal plasma components (Aquilina 2023; Mortimer 2020).

Ovarian stimulation protocol: The protocol of ovulation induction used for all women enrolled in this study was GnRH antagonist protocol by injection of Cetrorelix, 0.25mg, subcutaneous. It was started on day two or three of the menstrual cycle by the administration of 150-225 IU of the Recombinant FSH injection, subcutaneously once per daily.

Oocyte Retrieval: Oocyte aspiration was performed by gynecologist under general anesthesia by transvaginal ultrasound-guided, oocyte aspiration 34-36 hours after hCG administration (Oltedal et al. 2024). When the cumulus oocytes complexes were collected, they rinsed and washed with medium to remove residual blood from the aspirated follicles. Then quality of the cumulus-oocyte complexes were determined and stored in an incubator at 37°C, 5% CO_2, 95% humidity (Mugeir et al. 2022; Zarqaoui et al. 2020).

Oocyte Preparation: After oocyte collection, the cumulus coronal cells were stripped using 40 IU / ml hyaluronidase (Vitrolife / Sweden) and mechanically. Then the oocytes were assessed for maturation according to Palmerini et al. (2022)

Intra Cytoplasmic Sperm Injection Technique: The ICSI procedure was done by taking single sperm from prepared semen and injected it in the cytoplasm of mature oocyte by ICSI device. After ICSI procedure (16-18) hours, fertilization was assessed for the evidence of normal fertilization which was defined as the existence of two pronuclear and two polar bodies (Fischer et al. 2021).

Embryo Grading: The scoring of embryos were done after fertilization as the following: 26-28hrs for 2-cell (day 1) while 42-44 hrs the embryos were reached to 4 cells stage on (day 2), the embryo became 6-8 cells on day 3(66-68hrs) as described by Fischer et al. (2021).

The embryo grading system in the present study was depended on the Cummins' grading system (Cummins et al.1986) . The embryos were scored on a scale from 1 to 4:

Grade I: The excellent quality embryos that hade blastomeres (cells) with the equal size and no fragmentation were graded as G1(<10%). Grade II: Embryos are that had some minor asymmetry or minimal fragmentation, meaning that the blastomeres without perfect equal in size, and they had some small fragments were considered as GII (>10%). Grade III: Embryos with GIII had moderate asymmetry or fragmentation. This means that the blastomeres showed more unevenness in size, with a moderate amount of fragmentation (>25%). Grade IV: embryos have significant asymmetry or fragmentation GIV. This means blastomeres were showed a significant variation in size with substantial amount of fragmentation (>50%).

Fragment removal: Fragment removal was mainly performed in GIII and GIV embryos (degree of fragmentation >25%) on day 2 and 3 (Figure-1). As described by Kim et al (2018), fragment removal was performed by attaching an aspirator tube for calibrated microcapillary pipettes (A5177; Sigma-Aldrich, St. Louis, MO, USA) to a mouthpiece after attaching a micropipette with a 30-µM outer diameter to the pipette holder. Micromanipulation was performed in pre-warmed Petri dishes (351006; Corning, New York, NY, USA) in 30-µL droplets consisting of biopsy medium. Before fragment removal, the fragmented embryos were incubated in Ca^{2+}- and Mg^{2+}-free biopsy medium (Biopsy Media, Origio) under paraffin oil (Ovoil; Vitrolife, Sweden) for 30 minutes. The laser hole was made in the zona pellucida then the fragments around and between the blastomeres were sucked out by using hand-made micropipette with a mouthpiece from without touching or damaging the blastomeres. After complete fragment removal, the embryos were washed with culture medium and then incubated for an additional 24 hours for following up the further development of treated embryos**.**

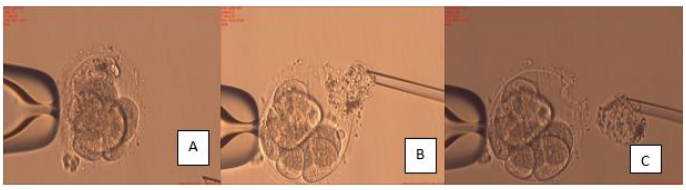

Figure -1: Show fragmented removal Before (A), During (B) and After(C). Magnification, X200.

Morphological status in embryos following elimination of fragments

The changes in the embryonic morphology after fragment removal were measured by embryo grade and developmental stage. The comparison in morphological changes of embryos after fragment removal and subsequent culture according to the embryo grade of the fragmented embryos was done.

Statistical Analysis: The values in the tables were presented as mean± standard error of the mean. The student *t*-test was employed to analyze differences the number of oocytes retrieved between the fragment removal cohort and the control group. Differences in clinical outcomes between the two groups were analyzed by the chi square test, and *p*-values <0.05 were considered to indicate statistical significance.

RESULTS

1. Baseline characteristics of all patients enrolled in the present study

Forty infertile couples were enrolled in the present interventional study, the results were expressed in mean ± standard error of the mean (SEM), the mean males' age was 39.03 ± 1.26 years and mean female age was 32.25 ± 0.85 years.

Regarding the causes of infertility, 9 (22.5%) couples were due to female causes, 22 (55.0%) couples because of male factors, 7 (17.5%) couples with unexplained causes and 2 (5.0%) couples with combined causes; furthermore only 9 patients (22.5%) were needed testicular biopsies in addition to the embryos fragmentation drilling of 17 (42.5%) patients done at day 2 embryos in contrast to 23(57.5%) patients was done at day 3 embryos.

Table -1: Baseline characteristics of all patients enrolled in the present study

Demographic features		Range	Mean ± SE
Female age (years)		23 - 45	32.25 ± 0.85
Male age (years)		27 - 54	39.03 ± 1.26
BMI (Kg/m²)		19.61 – 34.89	27.08 ± 0.59
Duration of infertility (years)		>1 - 21	9.05 ± 0.94
BMI ranking n. (%)	Normal weight	14 (35.0 %)	
	Over weight	15 (37.5 %)	
	Obese	11 (27.2 %)	
Causes of infertility	Female causes	9 (22.5 %)	
	Male causes	22 (55.0 %)	
	Unexplained	7 (17.5 %)	
	Combined causes	2 (5.0 %)	
Testicular biopsy N. (%)	Positive testicular biopsies	9 (22.5 %)	
	Negative testicular biopsies	31 (77.5 %)	
Day of embryo's fragmentation removal N. (%)	Day 2	17 (42.5 %)	
	Day 3	23 (57.5 %)	

SE: Standard Error; n: Number of patients; BMI: Body mass index

2 Comparison between studied groups according to the day of embryo's fragmentation removal

Although embryo growth was better at day 2 of fragmentation removal than day 3 (58.8% vs. 43.5%); there were no significant differences of embryos growth between day 2 and day 3 (p=0.337) as shown in table -2.

Table -2: Comparison between positive and negative embryos according to the day of embryo's fragmentation removal

Day of embryo's fragmentation removal	*Group 1 (Positive embryos) N.= 20*	*Group 2 (Negative embryos) N. =20*	*p value*
Day 2 N. (%)	*10 (58.8 %)*	*7 (42.2 %)*	*0.337 NS*
Day 3 N. (%)	*10 (43.5 %)*	*13 (56.5 %)*	

3 Comparison of ICSI characteristics among patients at day 2 and day 3 of fragmentations removal

All ICSI parameters demonstrating no significant differences between developed group 1 and non-developed group 2 embryos at day 2 of fragmentations removal (table -3). However, developed blastomere in group 1 at day 3 of fragmentations removal showed significantly lower grade III embryos count compared to non-developed blastomere in group2 (0.20 ± 0.13 vs. 0.62 ± 0.21; p=0.047) as presented in and table -4.

Table -3: Comparison of ICSI characteristics among patients at day 2 of fragmentations removal

ICSI parameters (Mean ± SE)	Group 1 (Positive embryos) N.=10	Group 2 (Negative embryos) N.=7	p value
Total oocytes count	18.70 ± 4.03	17.29 ± 4.99	0.827 Ŧ NS
Metaphase I oocytes	1.10 ± 0.28	0.86 ± 0.46	0.638 Ŧ NS
Metaphase II oocytes	14.20 ± 3.59	14.00 ± 4.60	0.973 Ŧ NS

ICSI parameters (Mean ± SE)	Group 1 (Positive embryos) N.=10	Group 2 (Negative embryos) N.=7	p value
Germinal vesicles	3.00 ± 0.83	2.43 ± 0.92	0.656 Ŧ NS
Abnormal oocytes	0.70 ± 0.39	0.43 ± 0.42	0.654 Ŧ NS
PN	10.00 ± 2.64	10.71 ± 3.58	0.871 Ŧ NS
Total embryos	10.00 ± 2.64	10.71 ± 3.58	0.821 Ŧ NS
Grade I embryos	2.50 ± 0.90	0.29 ± 0.18	0.061 Ŧ NS
Grade II embryos	3.60 ± 1.06	5.71 ± 2.66	0.418 Ŧ NS
Grade III embryos	3.30 ± 0.84	4.00 ± 1.18	0.626 Ŧ NS
Grade IV embryos	0.60 ± 0.34	1.00 ± 0.58	0.534 Ŧ NS

Ŧ: Independent sample t test; NS: Not significant (p > 0.05)

Table 4: Comparison of ICSI parameters among patients at day 3 of fragmentations removal

ICSI parameters (Mean ± SE)	Group 1 (Positive embryos) N.=10	Group 2 (Negative embryos) N.=13	p value
Total oocytes count	18.00 ± 4.94	12.46 ± 2.38	0.154 Ŧ NS
Metaphase I oocytes	1.10 ± 0.54	1.08 ± 0.27	0.968 Ŧ NS
Metaphase II oocytes	14.10 ± 2.41	9.85 ± 1.85	0.169 Ŧ NS
Germinal vesicles	2.60 ± 0.31	1.31 ± 0.50	0.053 Ŧ NS
Abnormal oocytes	1.00 ± 0.68	0.23 ± 0.17	0.232 Ŧ NS
PN	10.20 ± 1.64	7.23 ± 1.43	0.186 Ŧ NS
Total embryos	11.20 ± 2.02	7.23 ± 1.43	0.114 Ŧ NS
Grade I embryos	4.50 ± 1.11	2.85 ± 0.98	0.978 Ŧ NS
Grade II embryos	3.70 ± 0.62	2.15 ± 0.44	0.277 Ŧ NS
Grade III embryos	0.20 ± 0.13	0.62 ± 0.21	**0.047 Ŧ S**
Grade IV embryos	11.20 ± 2.02	7.23 ± 1.43	0.139 Ŧ NS

Ŧ: Independent sample t test; NS: Not significant (p > 0.05); S: Significant (p ≤ 0.05)

4 Comparison of embryos grading among all patients enrolled in the present study before and after fragmentations removal

There were no significant differences in embryonic development between before and after fragmentations removal in all patients enrolled. However, there was insignificantly increased in grade I and grade II embryos after fragmentations removal; (grade I = 20.73%;3.98 ± 0.39vs. 25.01%; 4.80 ± 0.39 ; p=0.064) and (grade II before=37.78%;7.25 ± 0.67vs. after=44.29%; 8.50 ± 0.67; p=0.841) respectively, on the contrary there were insignificantly lower grade III embryos (before=34.18%;6.56 ± 0.37vs. after= 24.49%; 4.70 ± 0.36 ; p=0.358) and grade IV embryos (before=7.29%;0.68 ± 0.17 vs. after=6.20%; 1.19 ± 0.15; p=0.634) as illustrated in table -5.

Table -5: Comparison of embryo grading among all patients before and after fragmentations removal*(no=40)*

Embryo grading	Fragmentations removal Percentage (Mean ± SE)		p value
	Before	After	
Grade I embryos =<10%	(20.73%)3.98 ± 0.39	(25.01%)4.80 ± 0.39	0.064 Ŧ NS
Grade II embryos =>10%	(37.78%)7.25 ± 0.67	(44.29%)8.50 ± 0.67	0.814 Ŧ NS
Grade III embryos =>25%	(34.18%)6.56 ± 0.37	(24.49%)4.70 ± 0.36	0.358 Ŧ NS
Grade IV embryos =>50%	(7.29%)1.40 ± 0.17	(6.20%)1.19 ± 0.15	0.634 Ŧ NS

Ŧ: Independent sample t test; NS: Not significant (p > 0.05); S: Significant (p ≤ 0.05)

5. Comparison of embryos grading among patients at day 2 and day 3 before and after fragmentations removal

At day 2 after fragmentations removal, there was insignificantly higher grade I embryos (Before= 14.24%;1.59 ± 0.59 vs. After=17.66%; 2.12 ± 0.77; p=0.621) and grade II embryos (Before= 40.30%; 4.47 ± 1.24 vs. After=46.08%; 5.53 ± 1.17; p=0.483); however, there were insignificantly lower grade III (Before= 37.58%, 4.18 ± 0.64 vs. After=29.9%; 3.59 ± 0.78; p=0.572) and grade IV embryos (8%;0.88 ± 0.32 vs. 6.36%;0.76 ± 0.30; p=0.773).

On the other hand embryos at day 3 of fragmentations removal showed higher grade I (Before=19.66%; 1.39 ± 0.39 vs. After= 22.25%; 1.78 ± 0.54; p=0.599), grade II (Before=39.30%; 2.78 ± 0.71 vs. After=44.63%;3.57 ± 0.74; p=0.476). However, elimination of fragmentation in grade III was insignificantly lower than before elimination (Before=33.70%;2.83 ± 0.39 vs. After=27.75%; 2.22 ± 0.37; p=0.157). The same result was shown in grade IV embryos (Before= 7.34% 0.52 ± 0.19 vs. After= 5.37%;0.43 ± 0.14; p=0.692) as presented in table -6.

Table -6: Comparison of ICSI parameters among all patients before and after fragmentations removal *(no=40)*

Embryo grading	fragmentations removal Percentage (Mean ± SE)		p value
	Before	After	
Day 2 of fragmentations removal			
Grade I embryos =<10%	(14.24%) 1.59 ± 0.59	(17.66%)2. 12 ± 0.77	0.621 Ŧ NS
Grade II embryos =>10%	(40.30%)4.4 7 ± 1.24	(46.08%)5. 53 ± 1.17	0.483 Ŧ NS
Grade III embryos =>25%	(37.58%)4.1 8 ± 0.64	(29.90%)3. 59 ± 0.78	0.572 Ŧ NS
Grade IV embryos =>50%	(8%)0.88 ± 0.32	(6.36%)0.7 6 ± 0.30	0.773 Ŧ NS
Day 3 of fragmentations removal			
Grade I embryos =<10%	(19.66%)1.3 9 ± 0.39	(22.25%)1. 78 ± 0.54	0.599 Ŧ NS
Grade II embryos =>10%	(39.30%)2.7 8 ± 0.71	(44.63%)3. 57 ± 0.74	0.476 Ŧ NS
Grade III embryos =>25%	(33.70%)2.3 8 ± 0.39	(27.75%)2. 22 ± 0.37	0.157 Ŧ NS
Grade IV embryos =>50%	(7.34%)0.52 ± 0.19	(5.37%)0.4 3 ± 0.14	0.692 Ŧ NS

Ŧ: Independent sample t test; NS: Not significant ($p > 0.05$); S: Significant ($p \leq 0.05$)

DISCUSSION

1. Baseline characteristics of all patients enrolled in the present study

The mean males' age was 39.03 ± 1.26 years and mean female age was 32.25 ± 0.85; as illustrated in table -1. The mean age of the women and their husband was 32/39 which is similar to other reports (Van Blerkom et al. 2011; Yang et al. 1998). Perhaps couples have spent too much time treating infertility using conventional methods before presented to specialized Center. In natural and conventionally treated cycles, as well as in ART cycles, age is a critical element influencing the likelihood of pregnancy (Osaikhuwuomwan et al. 2018).

The mean BMI of women enrolled in the study was 27.08 ± 0.59. Even the mean of BMI revealed overweight status but by this finding, BMI factor is discarded from being effective on the results of embryo fragmentation or development status following ICSI cycle.

Regarding the causes of infertility only 9 patients (22.5%) were needed testicular biopsies. The testicular biopsy was 9out of 40 cases which is (22.5%) high percentage than expected. It has been reported that testicular biopsy mainly performed for sperm harvesting in men with non-obstructive azoospermia, to be used for intracytoplasmic sperm injection (Dohle et al. 2012).

Data in table -1 stated that removal of fragmentation in embryos at day 2 done for 17 (42.5%) patients, embryos in contrast to 23(57.5%) patients was done at day 3 embryos. Therefore, the fragmentation starts in day 2 of 17 embryo and was detected in 23 embryos at day 3. It has been demonstrated that fragmentation removal at day 2 (Kim et al. 2019) or day3 (Chi et al .2010) subsequent development of embryos.

2. Comparison between studied groups according to the day of embryo's fragmentation removal

As shown in table -2, embryonic development was better after day 2 of fragmentation removal than day 3 (58.8% vs. 43.5%), even that no significant differences was reported. This result may be due to the following advantages that contribute to the greater safety of day 2 fragment removal compared to day 3 fragment removal as mentioned by kim et al. (2019). In day 2 embryos were larger and have fewer blastomeres and wide intercellular spaces. These features can reduce the time needed to remove the fragments and the exposure time outside the incubator, which may otherwise damage the embryos. Moreover, the cytoplasmic membrane of day 2 embryos seems to be more elastic and durable against micromanipulation for fragment removal than that of day 3 embryos. Therefore, early fragment removal on day 2 poses less risk of mechanical damage to embryos than fragment removal on day 3. Lastly, the continuation of *in vitro* culture for 24 hours after fragment removal on day 2 makes it more reasonable to select and transfer the embryos that show a higher grade and developmental capacity, without fragment reoccurrence, than is the case when embryos are directly transferred immediately after fragment removal on day 3 (Kim et al. 2019).

Most clinical ICSI characteristics parameters that mentioned in tables 3 and 4 after removal of fragmentation in day 2 and day 3 are similar. The only exception was increase in GIII embryos of non- developed (negative)embryos at day 3 compared to positive developed embryos after removal of fragmentation. This finding may be resulted from the presence of large cytoplasmic cellular fragments (>25%) may also have an impact in the spatial arrangement of the blastomere and they can cause apoptosis or the loss of a significant volume of cytoplasm, limiting the rate of blastomere cleavage because of the distortion of the blastomere division planes, leading to abnormal compaction, cavitation and blastocyst formation (Cecchele et al.2022).

3. Comparison of embryos grading before and after fragmentations removal

The results in table -5 related to the embryo grading after fragmentation removal of all patients involved in current study and in table-6 ,embryo grading development at day2 and day 3 after fragmentation removal .The data revealed that Grade I and Grade II embryos were insignificantly increase compared to before the removal of fragmentation .In contrast ,The Grade III and Grade IV embryos were shown insignificant reduction in fragmented embryos. Thus, the current work observed beneficial effects of fragment removal on embryonic development. The result of present study is similar to that reported by other author who performed fragment removal in day 3 embryos and observed a sustained reduction in fragmentation until day 5 (Keltz et a.2010). In the present study, fragment removal tend to significantly improved the morphological grade of the developed embryos compared to non-developed embryos in the fragment removal on day 3.

It has been accepted that GI and GII embryos have a higher implantation potential than GIII and GIV embryos. In fact, almost all embryos with fragments had a low grade and a reduced implantation potential (Ebner et al.2001; Van Blerkom et al.,2001), and the removal of fragments has been found to improve not only embryo development, but also pregnancy outcomes (Halvaei et al 2015; Chi et al.2010). Moreover, transfer after fragment removal in lower-grade embryos resulted in implantation and live birth rates comparable to those observed when GI embryos were transferred (Ketz et al.2006). This indicates that fragment removal may ultimately

Conclusion: The data of current study found a great benefit after elimination of fragmentations from cleavage embryos at day 2 and 3 after ICSI cycle resulted in a positive development of blastomere and embryonic development. At the same time because this work was done for the first time in Iraq, it is recommended to transfer and/ or cryopreserve the yielded embryos in future to obtain high percentage of pregnancy rates.

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Author Contribution: Kawa A. Faytha performed the study, and Prof. Dr. Saad S. Al-Dujaily supervised the work.

Conflict of Interest: The authors declare no conflict of interest.

Ethical Clearance: The study was approved by the Ethical Approval Committee.

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