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OBJECTIVE: Immature human oocyte (germinal-vesicle stage − GV) are capable of getting mature "in vitro", however the rates of fertilization and pregnancies as a result are low comparing with the maturated oocyte "in vivo". Usually, we only connect the nuclear maturation on the oocyte classification. Nevertheless, the cytoplasmatic development is important on the cellular activities too. The aim of this study is to compare the human oocyte maturation in two culture medium (P1 and TCP-19), through observation of the nuclear maturation (oocyte in metaphase II stage-MII) and of the cytoplasmatic maturation (cortical grain distribution − CG).

DESIGN: Prospective randomized study.

MATERIALS AND METHODS: A total of 97 oocytes in GV were collected from 29 patients after ovarian stimulation to IVF. The oocytes were denuded with 40 IU hyaluronidase (Type IV, Sigma, SA) and divided in two groups after randomized distribution: Group I (n:63) − oocytes were in vitro maturated in P1 medium (Irvine Scientific, USA) supplemented with 10% HSA (Irvine Scientific, USA); Group II (n:34) − oocytes were in vitro maturated in TCM-199 (GIBCO BRL − USA) supplemented with 10% HSA, hCG (0.5IU/ml), FSH (0.05mg/ml), estradiol (1.0mg/ml), pyruvato (0.2mM) and amicacin (70mg/ml). Oocytes were incubated up to 37oC in an atmosphere of 5% CO2 for 24−48h. During this period they were analysed due to the presence or not of the first polar corpuscule (MII oocyte). The cytoplasmatic maturation was confirmed after coloring with 10μg/ml Lens culinaris FITC conjugated: cytoplasmatic immaturity (cluster CG or transition from the center to the periphery), and cytoplasmatic maturity (CG located in the oocyte periphery). The nuclear maturation was confirmed after coloring with 10μg/ml Hoescht 33342. Results were analyzed by Fisher's Exact Test.

RESULTS: After culture most of the oocytes showed nuclear maturation (MII): 79.4% in P1 and 70.6% in TCM 199. However there were observed in cytoplasmic maturation only in 34.9% of oocytes cultured in medium P1 and in 41.2% of oocytes cultured in medium TCM199. The table below shows the results.

CONCLUSION: These results indicate that immature oocytes can resumption of meiosis (MII) independent the media and cumulus cells presence. Furthermore, nuclear maturation is not always followed by cytoplasmic maturation, since both treatments oocytes with characteristics of immaturity were seen. Considering that CG can be used as indicative of cytoplasmic maturation, CG staining could be allied to nuclear observation in order to ensure the efficiency of "in vitro" maturation protocols in human oocytes.

Supported by: None.

Nuclear and Cytoplasmatic Maturation				
	Nuclear	maturation	Cytoplasmatic	maturation
	yes	no	yes	no
Group I	50	13	22	41
Group II	24	10	14	20
		p:0.45		p:0.66

P-368

Blastocyst formation and pregnancy outcome following embryo selection using a scoring system specific for day 3 embryos. B. Balaban, A. Isiklar, H. Gursoy, Y. Kilic, H. Bozdag, B. Urman. AMERICAN HOSPITAL OF ISTANBUL, Istanbul, Turkey.

OBJECTIVE: The purpose of this study was to evaluate the performance of a specific scoring system proposed by Desai et al for prediction of blastocyst formation as well as pregnancy and implantation rates.

DESIGN: Prospective case series.

MATERIALS AND METHODS: The new day 3 embryo scoring system in a modified form was evaluated in 120 blastocyst transfer cycles. a. Each embryo was scored according to their blastomere number and fragmentation pattern. If fragmentation was more than minimal fragments in association with one blastomere, two points were subtracted from the blastomere number to give the embryo score. b. Additional 0.4 points were added to the final score in the presence of *(i)* good blastomere expansion, *(ii)* equal cell size, *(iii)*absence of vacuoles, *(iv)* cytoplasmic pitting and *(v)* signs of compaction. The final score was obtained by (blastomere number) + (-2 for high fragmentation) + 0.4 (each morphological feature). Patients were subdivided into 6 groups according to the score presented by more than half of the observed embryos.

RESULTS: The groups were comparable regarding female age and number of oocytes retrieved. The blastocyst quality was extremely poor in groups A-C as well as the clinical pregnancy and implantation rates. Blastocyst formation rate was higher than 50% for embryos with scores >7. Higher embryo score on day 3 was associated with increased rate of Grade 1 and 2 and hatching blastocyst formation on day 5. In group F, approximately 75% of blastocysts transferred were either Grade 1 or Grade 2 and >50% were hatching.

	Patients with >50% of observed embryos with					
Blastocyst transfer cycles	Score <5; Group A	Score 5-5.9; Group B	Score 6-6.9; Group C	Score 7-7.9 Group D	Score 8-8.9 Group E	Score >9 Group F
No. cycles	12 (10.0)	19 (15.8)	28 (23.3)	30 (25.2)	18 (15.0)	13 (10.8)
No. oocytes retrieved (mean)	125 (10.4)	194 (10.2)	289 (10.3)	306 (10.2)	186 (10.3)	137 (10.5)
2PN fertilization (%)	74 (74.7)	98 (70.0)	163 (75.1)	154 (70.0)	99 (71.7)	72 (72.7)
Cleaved embryos (%)	73 (98.6)	97 (98.9)	162 (99.3)	150 (97.4)	98 (98.9)	71 (98.6)
Blastocysts (%)	13 (17.8)	21 (21.6)	60 (37.0)	73 (48.6)	55 (56.1)	46 (64.7)
Hatching blastocysts (%)	0	0	9 (15.0)	19 (30.1)	18 (50.0)	13 (52.0)
No. blasts transferred (mean)	13 (1.0)	21 (1.1)	60 (2.1)	63 (2.1)	36 (2.0)	25 (1.9)
BG1+BG2 blasts transferred (%)	3 (23.0)	6 (28.5)	22 (36.6)	34 (53.9)	26 (69.4)	19 (76.0)
Clinical pregnancy (%)	2 (16.6)	5 (26.3)	11 (39.2)	17 (56.6)	12 (66.6)	9 (69.2)
Implantation (%)	2 (15.3)	5 (23.8)	16 (26.6)	26 (41.2)	19 (52.8)	14 (56.0)

CONCLUSION: The decision to prolong the in-vitro culture until the blastocyst stage is usually made according to the number of good quality embryos on day 3. The conventional grading system takes into account the blastomere number, fragmentation rate and equality of blastomere size. The addition of morphological criteria and fragmentation pattern into the conventional grading system may increase the power for prediction of embryos with the highest potential to develop into blastocysts that will yield optimum implantation rates.

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P-369

Efficacy of fresh and thawed testicular sperm using ICSI in azoospermic patients. Y.-S. Park, S.-H. Lee, H. K. Byun, J. H. Jun, M. K. Koong, J. T. Seo. Samsung Cheil Hospital, Seoul, Republic of Korea.

OBJECTIVE: Assessed the efficacy of cryopreservation of testicular sperm obtained from azoospermic patients and also compare the cycle outcome following ICSI with testicular sperm from frozen-thawed seminiferous tubule.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: In 776 patients, a total 1242 cycles of TESE was performed. Obstructive azoospermia was 680 cycles (54.8%) and non-obstructive azoospermia was 562 (45.2%) cycles. In TESE cycle, remained seminifeous tubules were cryopreserved after sperm preparation. The sections of seminiferous tubules were mixed in human semen preservation medium. And the ampules containing sample were frozen by programmed cell freezer (CryoMagic I, Seoul, Korea). For thawing, frozen samples were placed at room temperature and washed to remove cryoprotectant and sperm were retrieved in seminiferous tubule.

RESULTS: The total fertilization rates with 2-PN were 66.1% and pregnancy rates were 27.9% (347/1242). In obstructive azoospermia, fertilization rates with 2-PN were 68.4% and pregnancy rates were 33.4% (227/680). The pregnancy rates in fresh and thawed group were 35% (127/363) and 31.5% (100/317), respectively. In non-obstructive azoospermia, fertilization rates with 2-PN were 63.8% and pregnancy rates were 21.4% (120/562). In hypospermatogenesis (HS, n=257), total pregnancy rates of fresh and thawed cycles using patient's own sperm were 38.6% (98/254). And pregnancy rates in fresh group and thawed group were 25.6% (65/254) and 11.8% (30/254), respectively. In maturation arrest (MA, n=80), total pregnancy rates of fresh and thawed cycles using patient's own sperm were 20% (12/60). And pregnancy rates in fresh group and thawed group were 15% (9/60) and 5% (3/60),

respectively. In Sertoli cell only syndrome (SCO, n=188), total pregnancy rates of fresh cycles using patient's own sperm were 16.7% (10/60). In Klinefelter's syndrome (KS, n=37), total pregnancy rates of fresh cycles using patient's own sperm were 20% (3/20).

CONCLUSION: Optimal fertilization and pregnancy can be achieved using fresh- and thawed testicular sperm. Thawed testicular sperm showed reduced fertilization and pregnancy rates, cryopreservation of testicular sperm offers a suitable chance for azoospermic patients. Especially in non-obstructive azoospermic patients, although pregnancy rate was reduced than obstructive azoospermia, optimum pregnancy rate can achieved. Therefore, cryopreservation of seminiferous tubule is an effective procedure for optimal fertilization and pregnancy rates in azoospermic patients.

Supported by: None.

P-370

Can endometrial coculture be an alternative culture system for repeated ART failures? S. Sertyel, M. Aygun, H. Karagozoglu, F. Vanlioglu, G. Karlikaya, S. Kahraman. Istanbul Memorial Hospital, Istanbul, Turkey.

OBJECTIVE: Although in vitro fertilization techniques are now well established and being used worldwide, relatively lower success rates can partially be attributable to gamete and embryo quality as well as sub-optimal embryo culture conditions. Autologous endometrial coculture (AEC) is a recently proposed culture technique that can promote the development of embryos using patients own endometrial cells. In this study, a possible benefit of AEC on cases with at least two previously unsuccessful ART atempts were analyzed in 45 couples undergoing 48 cycles. In 23 of these cycles, embryo development parameters were also compared with that of previous cycles.

DESIGN: Retrospective study.

MATERIALS AND METHODS: Endometrial biopsy was taken between 19th-21st of the menstrual cycle. Biopsied sample was digested both mechanically and enzymatically and cells were cultured to a suitable density in 37°C with 5% CO_2. Zygotes having normal fertilization were transferred into coculture environment consisting of monolayers prepared from either fresh or frozen thawed endometrial cells. Embryos were kept in this system until embryo transfer.

RESULTS: 47 embryo transfers with AEC cycles resulted in 27.7% clinical pregnancy and 12.3% implantation rates respectively. One embryo transfer was canceled due to cleavage stage arrest. When embryo development was further compared with that of previous cycles with conventional medium, AEC system was found to improve embryo development and blastocyst formation in extended culture as well as decrease cytoplasmic fragmentation in cases with slow embryo development. No additional benefit of AEC on emrbyo development was found in cases with slow embryo development together with high cytoplasmic fragmentation.

Table. Embryo development profile in coculture cycles and previous cycles with conventional media for 23 patients.

	In Coculture (n,%)	In Conv. Media (n,%)
Day 1 (n)	165	251
Group I	148 (89,7)	229 (91,2)
Group II	17 (10,3)	22 (8,8)
Day 2 (n)	165	251
PN-stage arrested	11 (6,7)	24 (9,6)
2 cells	50 (30,3)	71 (28,3)
3-4 cells	104 (73,0)	156 (62,1)
Day 3 (n)	154	227
Arrested	12 (7,8)	24 (10,6)
<6 cells	47 (30,5)	76 (33,5)
>6 cells	95 (61,7)	127 (55,9)
Day 4 (n)	132	156
Arrested	30 (22,7)	29 (18,6)
<10 cells	39 (29,5)	57 (36,5)
>10 cells	63 (47,7)	70 (44,9)
Day 5 (n)	41	64
Arrested	8 (19,5)	12 (18,8)
Compaction/morula	12 (27,3)	46 (71,9) (*)
Cavitating/Blastocyst	21 (51,2)	6 (9,4) (*)

(*): p<0,01

CONCLUSION: Although exact mechanisms are still poorly understood, these results may imply that autologous endometrial culture systems can exert its beneficial effect by improving embryo survival and implantation. Therefore, where available, blastocyst stage embryo transfer programme in combination with autologous endometrial coculture can be an effective approach to increase the overall ART performance in such cases.

Supported by: None.

P-371

Does group culture of human embryos enhance day 3 embryo quality, implantation rates and pregnancy outcome? X. Yang, S. Shen, V. Y. Fujimoto, M. I. Cedars. Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, CA.

OBJECTIVE: It has been suggested that culturing embryos in groups may be beneficial because autocrine and paracrine growth or survival factors produced by the embryo may support other embryos. However, the literature is conflicting as to whether group culture truly enhances early human embryo development. Furthermore, very limited published data is available regarding the appropriate or optimal number of embryos to group. The aim of this study was to evaluate embryo quality when varying numbers of embryos were cultured together and to re-assess the overall benefit of group culture as determined by implantation rate and pregnancy outcome.

DESIGN: Retrospective review.

MATERIALS AND METHODS: A total of 550 IVF/ICSI cycles (4713 embryos) from the UCSF IVF program were analyzed (from period of 2000 to 2003). Co-cultured embryos were excluded from this study. Immediately following documentation of fertilization, embryos were either cultured individually or by grouping in different numbers. All embryo culture was in 25 ul of P1 medium supplemented with 10% SSS, and kept in 5% CO2 incubator until day 3. The embryos were assessed on day 3 for cleavage and fragmentation. Transfers were all performed on D3 after embryo evaluation. Clinical pregnancy was defined as yolk sac on ultrasound at six weeks of gestation. Statistical analysis was performed by ANOVA or Chi-Square test.

RESULTS:

Table 1. D3 Embryo Quality Among Different Number Of Groupings

# Of Embryos Per Drop	Patients Age	Total Embryos	6 Cells And Above, With fragmentation < 25%	6 Cells And Above, With fragmentation >25%	5 Cells And Under
1	35.0 ± 5.1	900	561 (62%)	99 (11%)	240 (27%)
2 To 5	34.8 ± 5.2	1234	748 (61%)	95 (8%)	391 (31%)
6 To 9	34.7 ± 5.3	1904	1227 (64%)	127 (7%)	550 (29%)
≥ 10	34.0 ± 5.5	675	406 (60%)	70 (10%)	199 (29%)
	NS		NS	NS	NS

Table 2. Implantation And Clinical Pregnancy Rate-Comparison Of Group With Non-Group Culture Group Non-Group

# Cases	403	115	
Average Embryos #	6.2 ± 2.9	6.8 ± 3.8	NS
Average Patients Age	34.6 ± 5.3	35.0 ± 5.0	NS
Average Embryos / ET	3.1 ± 1.2	3.3 ± 1.2	NS
Implantation Rate	21.0%	21.3%	NS
Clinical Pregnancy Rate	47.3%	50.4%	NS

Varying the number of embryos, in a 25 μl drop, for culture had no effect on day 3 embryo quality (P>0.05). Neither group nor non-group culture affects implantation and clinical pregnancy rates.

CONCLUSION: When culturing human embryos individually or in different groups from D1 to D3 in 25 μl of culture medium, no differences in D3 embryo cleavage and fragmentation score, implantation rates or pregnancy outcome were observed. This data provides assurance that both group culture and non-group culture can be utilized in IVF laboratory without compromising clinical outcomes.

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