P-252

Degree of re-expansion of thawed blastocysts is predictive of frozen embryo transfer (FET) success. D. C. Merryman, S. E. Stringfellow, C. A. Yancey, V. L. Houserman, C. A. Long, K. L. Honea. ART Program of Alabama, Birmingham, AL.

OBJECTIVE: Cryopreservation of excess embryos is routinely performed at the blastocyst stage. The number of blastocysts to thaw for transfer during an FET cycle is sometimes difficult to assess due to the immediate appearance of the thawed blastocyst. Frozen blastocysts contract after thawing and subsequently re-expand over time. The objective of this study was to evaluate the quality of frozen-thawed blastocysts at two hours after thawing according to the degree of re-expansion.

DESIGN: The current study is a retrospective analysis of the relationship of the degree of re-expansion of thawed blastocysts at two hours after thawing to ongoing pregnancy and/or delivery rate (ODR), defined as the number of ongoing pregnancies and/or deliveries per embryo transfer, and implantation rate (IR), defined as the number of ongoing fetal heartbeats and/or live births per number of embryos transferred. Pregnancies and fetal heartbeats were considered ongoing at 12 or more weeks' gestation.

MATERIALS AND METHODS: The data consisted of 25 FET cycles in which a two-hour picture of frozen-thawed blastocysts was available. Blastocysts were frozen and thawed utilizing glycerol and sucrose solutions. After thawing, blastocysts were cultured for a minimum of 2 hours before transfer. At two hours after thawing, the blastocysts were evaluated for degree of re-expansion. Fisher's exact test or Student's *t*-test was used for statistical analysis as indicated. Statistical significance was defined as $P < 0.05$.

RESULTS: FETs were grouped according to the number of blastocysts transferred with 50% or more re-expansion two hours after thawing. An increase in ODR and IR is shown when one or more blastocysts transferred were 50% or more re-expanded two hours after thawing (see table below). No ongoing and/or delivered pregnancies occurred when all blastocysts transferred were less than 50% re-expanded two hours after thawing.

CONCLUSION: IR and ODR can be optimized for FET cycles by evaluating the re-expansion of thawed blastocysts two hours after thawing. Thawing of excess frozen blastocysts may be avoided by initially thawing the minimum number to be transferred followed by a two hour assessment and only then thawing more if needed.

Treatment outcome as a function of blastocyst re-expansion in a group of 25 FET cycles.

	Number of blastocysts transferred with re-expansion to 50% or more two hours after thawing					
	0	1	2	3	4	≥ 1
Embryo transfers	5	8	7	4	1	20
Embryos transferred (avg/ET)	13 (2.6)	16 (2.0)	17 (2.4)	14 (3.5)	4 (4.0)	51 (2.6)
Ongoing fetal heart beats and/or live births (IR)	0 (0)	3 (18.8)	1 (5.9)	5 (35.7)	1 (25.0)	10 (19.6)
Ongoing and/or delivered pregnancies (ODR)	0 (0)	3 (37.5)	1 (14.3)	3 (75.0)	1 (100)	8 (40.0)
Age (avg/ET)	31.2	30.1	31.3	30.3	34	30.8

Supported by: None

P-253

Day 5 vs. day 6: A Comparison between fresh and frozen blastocysts transfer cycles. N. Zaninovic, L. L. Veeck, R. Bodine, R. Clarke, E. Manheim, Z. Rosenwaks. Weill Medical College of Cornell University, New York, NY.

OBJECTIVE: Blastocyst transfer (BL-ET), as opposed to day 3 preembryo transfers, represents a beneficial treatment for IVF patients because it allows for optimal embryo selection, which in turn results in high pregnancy (PR) and implantation (IM) rates with a concomitant reduction in the incidence of multiple gestations (only one to two blastocysts transferred). One of the main questions is if there is a significant difference in developmental potential of day 5 (D5) blastocysts as compared to day 6 (D6). Further, if such a difference exists, is it also reflected when comparing D5 and D6 frozen/thawed cycles? To answer these questions and determine which day, if either, is most advantageous for preembryo transfer (ET), we compared D5 and D6 PR and IM outcomes in fresh and frozen BL-ET cycles.

DESIGN: A retrospective analysis of clinical PR and IM rates in 548 fresh BL-ET (D5 and D6) and 359 frozen BL-TR (frozen either D5 or D6) was performed. Patients were divided into groups based on transfers with fully developed blastocysts and transfers with only morulae (no blastocysts transferred). Patients cultured to D5 or D6 solely due to medical indications or slow development were kept as a separate group.

MATERIALS AND METHODS: IVF patients with an adequate number of good quality preembryos on day 3 were selected for BL-ET on D5 (n=532) or D6 (n=16). Blastocysts with good morphology were frozen on D5 (n=115 thawed cycles) or D6 (n=225 thawed cycles). The patient groups were subdivided as follows: D5 fresh, with blastocysts transferred (n=496) or morulae transferred (n=36); D6 fresh (15/16 with blastocysts transferred); D5 frozen; D6 frozen; medical/slow. Statistical analysis was performed using Fisher or Chi Square tests.

RESULTS: Clinical PR and IM rates were significantly higher in fresh BL-ET as compared to frozen/thawed: fresh BL-ET cycles (including both D5 and D6) demonstrated 68% clinical PR vs. 49% frozen/thawed, and 52% IM vs. 23% frozen/thawed rates ($P=0.0001$). Taken independently, the D5 and D6 fresh cycles showed no significant differences for clinical PR (69% vs. 56%) or IM (52% vs. 43%). Significantly higher PR and IM rates were observed in patients with transferred blastocysts as opposed to transferred morulae in fresh D5 cycles (71% vs. 33% and 56% vs. 17% respectively, $p=0.0001$). In addition, patients with only morulae on D5 achieved greater clinical PR results (56% vs. 33%, NS) and significantly better IM rates (43% vs. 17%, $p=0.0006$) if they were left in culture and transferred on D6 (D6 blastocyst vs. D5 morula/ET). There were no differences between D5 and D6 frozen/thaw cycles for PR (50% vs. 48%) or IM (23% vs. 23%). Patients undergoing D5/D6 ETs due to medical indications or slow preembryo development (not selected for BL-ET by virtue of good growth rate and morphology) had poor outcomes, with only an 8% PR rate and 6% IM rate.

CONCLUSION: Blastocyst transfer, whether fresh or frozen, represents a good treatment option for IVF patients, although fresh BL-ET did demonstrate better pregnancy and implantation results as compared to frozen/thawed. Overall, we observed no differences between D5 and D6 fresh ET. In addition, cryopreservation of blastocysts on D5 or D6 showed no differences in pregnancy and implantation rates in subsequent thawed cycles. Finally, we recommend that preembryos with only morulae on day 5 be left in culture an additional day; this allows for improved blastocyst selection, which then results in a higher rate of pregnancy and implantation.

Supported by: None

P-254

Comparison of human tubal fluid (HTF), G1.2®, and Sage Cleavage® media for human *in vitro* fertilization (IVF). V. W. Aoki, A. L. Wilcox, K. Parker-Jones, H. H. Hatasaka, M. Gibson, D. T. Carrell. University of Utah, Salt Lake City, UT.

OBJECTIVE: The aim of this study was to compare the effects of human tubal fluid (HTF), G1.2®, and Sage Cleavage® media on in-vitro fertilization (IVF) embryo quality and outcome during three-day culture of human embryos.

DESIGN: A two-phase study in which, initially HTF and G1.2® were compared followed by comparison of HTF and Cleavage® Medium for IVF outcome.

MATERIALS AND METHODS: The study was conducted in two phases. The first phase involved 121 consecutive IVF cases in which half the embryos from each patient were cultured in HTF medium (n = 411) while the other half were cultured in G1.2® medium (n = 369). The second phase involved 553 consecutive IVF cases where half the embryos from each patient were cultured in HTF medium (n = 1,615) and the other half were cultured in Sage Cleavage® medium (n = 1,867). Embryos were transferred on day three. Embryo quality (ES) was calculated by subtracting the embryo grade from the number of blastomeres in the embryo. Embryo grade is on a scale of zero to four and was assigned based on a combination of blastomere regularity and cellular fragmentation with zero being the best and four the worst. ES and pregnancy rates were evaluated retrospectively. Embryo quality and number were evaluated statistically using a paired T-test while pregnancy rates were evaluated using Chi-square analysis.

RESULTS: In phase-1 of the study embryo quality was not significantly different for embryos cultured in HTF versus G1.2® (3.95 ± 0.11 vs. 3.78 ± 0.11; $p = 0.25$). However, embryos derived from ICSI displayed significantly improved quality when grown in HTF (4.28 ± 0.14, n = 219)

versus G1.2® (3.73 ± 0.15, n = 199; $p < 0.01$). Embryos derived from normal IVF exhibit no difference in quality when grown in HTF (3.59 ± 0.18, n = 162) versus G1.2® (3.72 ± 0.17, p = 0.17). Pregnancy rates in cases where only HTF-cultured embryos were transferred (46%, n = 26) were slightly higher than those cases where only G1.2®-cultured embryos were transferred (36%, n = 11; p = 0.72). In addition, clinical pregnancy (31% vs. 33%) and implantation rates (25% vs. 21%) were virtually identical for HTF and G1.2®, respectively (p = 0.86). In phase-2, embryo quality was significantly improved for embryos cultured in Cleavage® versus HTF media (3.67 ± 0.06 vs. 3.48 ± 0.07; $p < 0.01$). Although ICSI cases did not reflect this difference (3.63 vs. 0.08 vs. 3.50 ± 0.09, p = 0.18) normal IVF cases showed significantly improved embryo quality with Cleavage® over HTF media (3.23 ± 0.09 vs. 2.91 ± 0.1, $p < 0.005$). The number of Cleavage® medium-grown embryos transferred per case was significantly higher than the number of HTF-grown embryos (1.51 ± 0.03 vs. 1.04 ± 0.03, $p < 0.001$). Additionally, the number of cases in which all embryos transferred were from the Cleavage® medium group was significantly higher than the number of cases in which all HTF-grown embryos were transferred (115 vs. 34; $p < 0.001$). However, chemical pregnancy, clinical pregnancy, implantation, and spontaneous abortion rates were similar for these cases.

CONCLUSION: Overall, the data indicates Cleavage® medium is superior to HTF and G1.2® media for culture of embryos during human IVF as it results in significantly improved embryo quality versus HTF medium. Meanwhile, HTF medium shows similar embryo quality to G1.2® with similar pregnancy and implantation rates.

Supported by: None

P-255

Proportional oocyte nuclear maturation in relation to ICSI outcome. K. Baek, Q. V. Neri, A. Wang, T. Takeuchi, Z. Rosenwaks, G. D. Palermo. Cornell University, New York, NY.

OBJECTIVE: Depending on different stimulation protocols and varying patient response, about 70–80% of the oocytes are metaphase II at egg retrieval. When the contribution of the immature oocytes is higher, particularly when over 50%, this is cause of great distress to physicians and patients. In standard in vitro fertilization, the proportion of mature oocytes can be determined only on the day of fertilization assessment, whereas in the case of ICSI this becomes obvious immediately after cumulus removal, allowing insemination of only mature oocytes. In the present study, we aimed to assess the impact of a high proportion of immature oocytes present at retrieval on ICSI outcome.

DESIGN: Patients who underwent the ICSI procedure were classified retrospectively according to the proportion of immature oocytes at the time of retrieval. Term outcomes were fertilization, implantation, and pregnancies.

MATERIALS AND METHODS: Couples treated by ICSI from September 1993 to December 2001 were included in this study. In order to exclude confounding factors related to maternal age or severe male factor, only women ≤35 years of age and only ejaculated spermatozoa were used. Ovarian stimulation was performed with a GnRH-agonist/antagonist and gonadotropins, with oocytes retrieved approximately 36 hours after hCG administration. ICSI was then performed only on MII oocytes in a standard fashion. Fertilization was assessed 16–18 hours after insemination and good quality embryos were transferred to the patients either on day 3 or day 5. Patients were stratified into three groups: with 100% maturation (complete), those with 50% of the oocytes matured (partial), and those with only 30% mature (minimal).

RESULTS: A total of 2,368 ICSI cycles using ejaculated spermatozoa were assessed, with a similar maternal age in all groups and an overall average maternal age of 32.1 ± 3 yrs (range 18–35 yrs). The number of cycles for the complete maturation group was 388 (16.4%), for the partial maturation was 116 (4.9%), and for the minimal maturation was 31 (1.3%). The total number of oocytes retrieved was 3,274, 624, and 87, respectively, while the average number of oocytes injected was 8.4, 5.5, and 3.9. The fertilization rates and patterns were not different among the three cohorts, being 73.5, 69.7, and 76.9%, respectively, and the implantation rate was directly related to number of mature oocytes (28.9, 31.8, and 23.8%). Proportional differences were found in the pregnancy (46.4, 50.0, and 29.0%; $P < 0.01$) and in delivery rates (44.3, 44.8, and 25.8%; $P < 0.01$). Thus, while the outcomes for the complete and partial maturation gorups were comparable, the minimal maturation group had a lower rate of success.

CONCLUSION: Within the cohort of oocytes retrieved the proportion of those at the MII stage did not affect fertilization outcome as long as at least half of them were mature. Only when ~33% of mature oocytes was present was when the ICSI outcome compromised. Although the lower number of mature oocytes did not impair quality of any embryos eventually produced as judged by the rates of implantation, this still impairs pregnancy outcome because of a lower number of embryos transferred.

Supported by: Institutional.

P-256

Blastocyst transfers: Is there any benefit to transfer more than 2 embryos? A. R. Anderson, J. Whelan, J. L. Crain. Institute for Assisted Reproduction, Charlotte, NC; Reproductive Endocrine Associates of Charlotte, Charlotte, NC.

OBJECTIVE: The objective of this study was to determine if there is any added benefit to transfer more than two blastocysts based on embryo morphology.

DESIGN: Retrospective analysis of blastocyst embryo transfers between 1/1/02 and 12/31/03 where either one or two embryos were transferred compared to three embryos.

MATERIALS AND METHODS: All blastocysts were evaluated for degree of blastocoel cavity and expansion on day 5. Expanded blastocysts were considered the optimal embryo for transfer. Data was analyzed based on the number of expanded blastocysts present in the transfer procedure.

RESULTS: When all embryos available for transfer were expanded blastocysts were transferred 89/157 (57%) of the embryos implanted. In two embryo transfers, where only one embryo was expanded, 70/166 (42%) implantation rate was achieved. Where there were no expanded blastocysts available for transfer the implantation rate was only 101/329 (31%). For 3 embryo transfers there were no patients with all expanded blastocyst in the study population. In three embryos where at least one embryo was expanded blastocyst available for transfer the implantation rate was 10/21 (47%). In three embryo transfers and no expanded blastocysts available for transfer the implantation rate was 16/72 (22%) respectively. There was no significant improvement in implantation when 3 embryos were transferred in the three subgroups. There was a significantly ($P<0.001$) higher implantation rate where all embryos were expanded compared to no expanded or some expanded embryos. There was also a significantly ($P=0.012$) higher implantation rate where at least on embryo was expanded compared to no expanded embryos available for transfer.

CONCLUSION: Number of expanded blastocysts available at transfer is considered a gold standard. However in this study we have shown no added benefit to increasing the number of embryos if there were no expanded embryos available for transfer. The presence of two or more expanded blastocysts available for transfer may warrant consideration of a single embryo transfer.

Supported by: None.

Table 1: Implantation where one or two vs. three embryos transferred.

Embryo Quality	Implantation where 1 or 2 embyros transferred	Implantation where 3 embryos transferred	Significance
All expanded blastocysts ET	89/157 (57%)	None performed	N/A
Some expanded blastocyst ET	70/166 (42%)	10/21 (47%)	NS
No expanded blastocysts ET	101/329 (31%)	16/72 (22%)	NS

P-257

Does intracytoplasmic sperm injection effect blastocyst development? A. R. Anderson. Institute for Assisted Reproduction, Charlotte, NC.

OBJECTIVE: The objective of this study is to determine if intracytoplasmic sperm injection (ICSI) has any detrimental effect on blastocyst development when compared to conventional insemination (CI).

DESIGN: Retrospective analysis of in vitro fertilization cycles where half the oocytes were treated with ICSI and half with CI methods.