

ENHANCED EMBRYO DEVELOPMENT & BLASTOCYST UTILISATION

Achieved with a single-patient-per-chamber time-lapse incubator and single-step medium, compared to conventional culture methods.

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AIM

To determine if embryo development and the resultant blastocyst utilisation rate following embryo culture in a single patient-per-chamber time-lapse incubator system using single step medium are comparable with those following embryo culture in a conventional environment.

> INTRODUCTION

The advent of time-lapse imaging systems with a single patient-per-chamber incubator in addition to a universal single-step medium brings about the possibility of uninterrupted culture, thereby potentially reducing the impact of environmental changes on the embryo throughout the culture period. To our knowledge, there have been limited studies comparing embryo development in a time-lapse system using single-step medium compared to a conventional culture environment using sequential media¹⁻³. There are also efficiency gains to be gleaned as a result of using time-lapse incubators alongside single-step culture media which inevitably lead to reduced cost and thus, these can be considered if uninterrupted culture leads to comparable, or better, embryo development and clinical outcomes.



Moreover, the optimisation of culture media in general and in particular, the development of enhanced versions of single-step culture media offer increased potential to better support the embryo throughout the in vitro culture process. The inclusion of specific compounds within culture media products have potentially beneficial impacts on embryo development, especially when considering key components such as L-Carnitine, which is shown to have multiple advantages for developing embryos such as; increasing energy metabolism through facilitating the movement of fatty acids across the mitochondrial membrane; acting as a scavenger for damaging free radicals and; protecting against DNA damage by preventing mitochondrial dysfunction.⁴⁻⁶

The relatively recent optimisation of culture media, and especially the advent of novel, single-step formulations, has also drawn special attention to points which must be considered in more depth when culturing embryos for a longer period of time in one culture medium without replenishment. As an example, specifically referring to ammonium – a known toxin which can negatively impact embryo development and has been directly linked to the breakdown of glutamine – culture media manufacturers have reacted by including it in its stable, dipeptide form (alanyl-glutamine) that has been shown to degrade less rapidly⁷. Similarly, the presence of glycine in culture media is known to increase embryos' ability to react to small osmolality changes which occur more readily as the in vitro culture period is lengthened (by being imported via the GLYT1 transporter and therefore regulating internal osmolality) and hence, the most advanced single- step culture media include glycine at a suitable, tested concentration⁸.



> METHOD

This study was conducted across Genea clinics in Australia and included all 2PN's from non-PGD cycles cultured in either the conventional culture environment using MINC (Cook) with sequential media (Gems, Genea Biomedx), time-lapse incubation with sequential media (Gems, Genea Biomedx) or complete time-lapse culture environment using Geri with Geri Medium (Gems, Genea Biomedx). Utilisation refers to embryos at a blastocyst stage or greater which were deemed suitable by conventional morphological selection for transfer or vitrification (note that Geri's time-lapse morphokinetic capabilities were not utilised). This was a retrospective cohort study investigating embryo development and blastocyst utilisation of 5236 normally fertilised oocytes across three culture systems used at different time periods. There were 2816 2PN embryos cultured in the conventional culture environment from August 2015 to February 2016, 290 in the time-lapse incubation with sequential media group from April to July 2016 and 2130 in the complete time-lapse culture environment from August 2016 to February 2017. Results included embryos cultured following both IVF and ICSI.



> RESULTS

The results of the comparative study showed that there were increases in both the improvements in embryo development and the resultant utilisation rate when Geri Medium was used in a time-lapse incubator, thus offering undisturbed culture, compared to when using a conventional, sequential culture system. The results of the study are summarised in Table 1.

All Clinic: Fresh ART Cycles	MINC & Sequential Gems (Aug 2015–Feb 2016)			Geri & Sequential Gems (Apr–Jul 2016)			Geri & Geri Medium (Aug 2016–Feb 2017)		
	<38	≥38	Combined	<38	≥38	Combined	<38	≥38	Combined
Average 2PN age	32.58	40.96	35.62	33.26	41.16	36.72	32.44	41.23	35.81
OPU Rank	1.68	2.81	2.09	1.74	3.28	2.33	1.57	2.46	1.85
Number of 2PN cultured	1794	1022	2816	179	111	290	1465	665	2130
>7 cells Grade 1/2 on day 3	1171 (66.0%)	651 (65.3%)	1822 (65.8%)	114 (64.0%)	73 (68.9%)	187 (65.8%)	979 (67.0%)	430 (65.3%)	1409 (66.5%)
Blastocysts (Total) on day 5	661 (38.6%)*	239 (26.0%)*	900 (34.2%)*	74 (42.3%)	22 (21.4%)	96 (34.5%)	730 (51.8%)*	242 (38.3%)*	972 (47.6%)*
Blastocysts (Grade 1/2) on day 5	460 (26.9%)*	173 (18.8%)*	633 (24.1%)*	56 (32.0%)	19 (18.4%)	75 (27.0%)	580 (41.1%)*	170 (26.9%)*	750 (36.7%)*
Utilisation rate (%)	40.1%*	34.5%*	38.1%*	54.7%	41.4%	49.7%	52.8%*	44.8%*	50.3%*

Table 1: Summary of morphological assessments for embryos cultured in Geri Medium or Gems sequential media, utilising either the Geri or MINC incubator (* $p<0.01$)

As detailed in Table 1, the demographics of 2PN's between all groups were comparable in terms of 2PN age between the MINC & sequential Gems and Geri & Geri Medium groups (35.62 and 35.81 years, $p=0.446$), the MINC & sequential Gems and Geri & sequential Gems groups (35.62 and 36.72 years, $p=0.535$), and the Geri & sequential Gems and Geri & Geri Medium groups (36.72 and 35.81 years, $p=0.762$).

There was an increased number of total blastocysts on day 5 observed in all age groups when Geri Medium was used in the Geri incubator than when Gems sequential media was used in the Geri incubator or in the MINC incubator. Specifically when considering the combined age group, the percentage of total blastocysts was 34.2% and 47.6% in the MINC & sequential Gems and the Geri & Geri Medium groups, respectively, representing a statistically significant increase ($p<0.01$).

Furthermore, also in the combined age group, improved quality of blastocysts on day 5 was observed, with a higher percentage of grade 1 or 2 blastocysts in the Geri & Geri Medium group compared with the MINC & sequential Gems group, 36.7% and 24.1%, respectively. This was also a statistically significant difference ($p<0.01$). The results of blastocyst development are graphically presented in Figure 1.

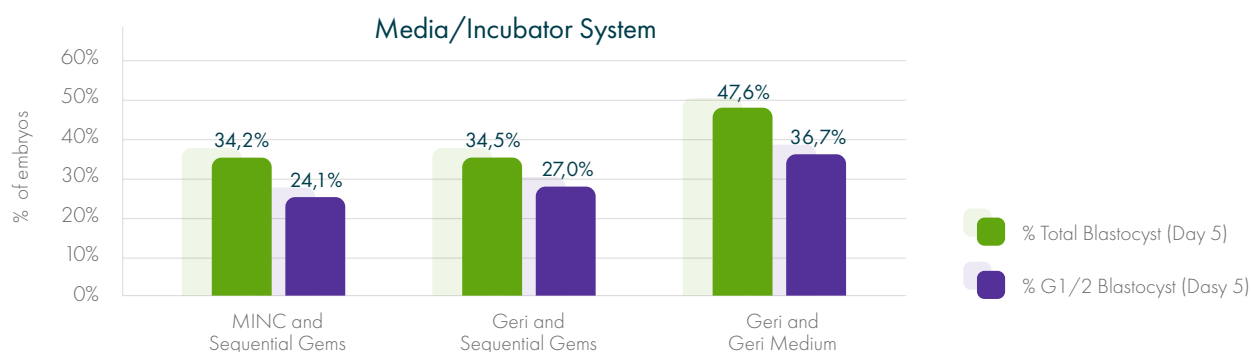


Figure 1: Results of blastocyst development in each culture system (combined ages)

Moreover, the utilisation rate of embryos cultured in the Geri & Geri Medium group was higher than that seen in both the Geri & sequential Gems group, and in the MINC & sequential Gems group. In particular, the utilisation rate observed in the Geri & Geri Medium group was 50.3% and in the MINC & sequential Gems group, it was 38.1%. This represents a statistically significant difference ($p < 0.01$). The utilisation rates of embryos in each of the culture systems is graphically presented in Figure 2.

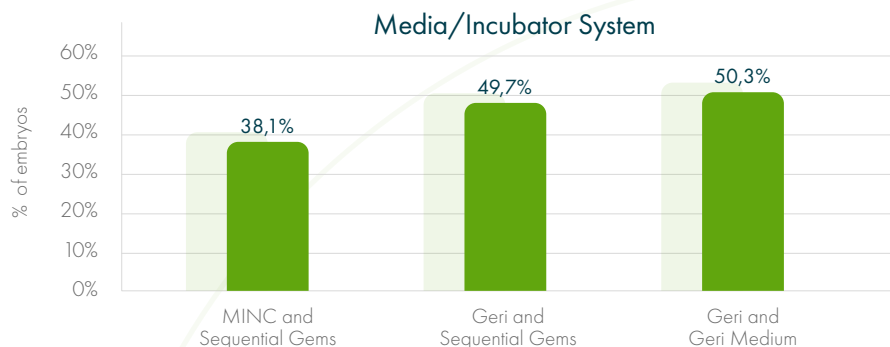


Figure 2: Utilisation rate of embryos cultured using each system (combined ages)

CONCLUSION

This study revealed improved embryo development and utilisation rates using a single patient-per-chamber time-lapse incubator system with single-step medium as compared with using the same time-lapse system with sequential media and with conventional culture, using a bench-top incubator with sequential media. This suggests that minimising interruption of embryos improves clinical outcomes and therefore, highlights the importance of undisturbed in vitro culture.

> LIMITATIONS, REASONS FOR CAUTION

This was a retrospective analysis. As the analysis compared culture methods performed at different time periods, it is possible that other factors also influenced results. Prospective studies are required to confirm these findings.

> REFERENCES

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> COMPETING INTEREST

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