

Statistical analysis plan:

Impact of Repeated Antioxidant Supplementation of Embryo Culture Media on Blastocyst Utilization and Expansion Rate Under Two Different O₂ Concentrations

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Research Questions:

- What is the effect of antioxidant supplementation and O2 tension on blastocyst utilization rate and blastocyst expansion rate in infertile women?
- Are there any significant differences in the outcomes between the different antioxidant supplementation protocols and O2 tensions?

Statistical Tests:**Sample Size:**

The total number of blastocysts analyzed was 1797, obtained from 3603 zygotes and 4924 inseminated oocytes. The study included 293 infertile women with a mean age of 32.2 ± 3.3 years.

Data Handling:

For statistical analysis, the data collected consisted of the continuous variable of age, which was expressed as the mean \pm standard deviation in each study group. In addition, the frequency and percentage of usable and expanded blastocysts were recorded as categorical variables.

Comparisons between treatments were performed by calculating the odds ratio and the chi-square test. A significance level of $p < 0.05$ was established to determine the existence of statistically significant differences between groups.

Confounding Variables:

Other treatments or medications: Although there is no explicit mention of other treatments or medications received by the participants in addition to antioxidant supplementation, it is essential to acknowledge that there may be confounding variables present. It is possible that other interventions or medications used in the management of infertility could interact with antioxidant supplementation and potentially influence the study outcomes.

Sensitivity analysis:

The odds ratio analysis with a confidence interval of 95%

9. Statistical Software:

R Core Team was employed for statistical analysis.

10. Data Interpretation:

The results were interpreted by analyzing and comparing the blastocyst utilization rates, blastocyst expansion rates, and cumulative blastocyst utilization and expansion rates across the four study

groups. Statistical analyses, such as odds ratios (OR) and chi-square tests, were used to determine the significance of the differences observed. The level of significance was set at $p < 0.05$.

The findings indicated that antioxidant supplementation had a positive impact on the development and usability of blastocysts. The groups receiving antioxidant supplementation every 12 hours (groups 1A and 2A) consistently showed higher blastocyst utilization and expansion rates compared to the groups with antioxidant supplementation only at the beginning of embryo culture (groups 1B and 2B). These results suggest that regular and sustained antioxidant exposure during embryo development may enhance the overall quality and viability of blastocysts.

Additionally, subgroup analyses were conducted based on the O₂ tension (20% vs. 5%) and the timing of antioxidant supplementation (every 12 hours vs. baseline only). These subgroup analyses allowed for a more detailed understanding of the effects of different conditions on blastocyst outcomes. The subgroup analyses consistently demonstrated that the groups with regular antioxidant supplementation every 12 hours (groups 1A and 2A) had superior blastocyst utilization and expansion rates compared to the groups with baseline-only supplementation (groups 1B and 2B).

In terms of clinical or practical significance, the findings suggest that incorporating antioxidant supplementation, particularly with a frequent administration schedule, can improve the development, expansion, and overall utilization of blastocysts in assisted reproductive technologies. This may have important implications for infertility treatments, as higher blastocyst quality and viability are associated with increased chances of successful embryo transfer and higher pregnancy rates. By optimizing embryo culture conditions through antioxidant supplementation, clinicians and embryologists can potentially improve clinical outcomes and increase the success rates of infertility treatments, offering hope to couples struggling with fertility issues.