Mathematically Adjusting for Embryo Size Improves the Accuracy of a Specific Gravity Device in Predicting Embryo Sex

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Abstract

Objective: Previous research from this laboratory has demonstrated a Specific Gravity Device (SGD) can be used to noninvasively assess embryo quality across the early stages of embryo development (zygote to blastocyst). Preliminary data also appears to suggest the system might also be useful in predicting embryo sex as embryos enter the blastocyst stage. However, as the SGD calculations assumed embryos of a consistent size, its usefulness was limited in expanding blastocyst. The objective of the current study was to determine if a corrective mathematical algorithm, based on embryo size, could enhance the ability of the SGD to predict embryo sex.

Design: Lab-based trial of the SGD in predicting embryo sex.

Materials and Methods: In this preliminary, a proprietary algorithm was developed to account for embryos of varying sizes based upon the physics of shape and buoyancy. Data from a previous experiment where 463 bovine blastocyst stage embryos' had SGD results correlated to sex determination by Polymerase Chain Reaction (PCR) were first corrected for size using the new mathematical algorithm and the embryo's predicted growth (expansion) from the time of blastocyst formation and exposure to the SGD, and then reanalyzed using the SGD prediction for sex. Finally, these new outcomes were compared to the PCR results to determine if correcting for embryo size improved the predictive power of the SGD.

Results: Initial studies without the size correction demonstrate the SGD 65.3-78.4% accuracy selecting for female embryos with no predictive power for male embryos. With the size adjustment, the predictive power of both was increased. Female to over 80% and male to over 60%. While further refinement of the mathematical adjust may be necessary, These data continue to suggest the SGD might provide a noninvasive means of predicting the sex of preimplantation embryos.

Conclusions: Theoretically, the differences in the buoyancy of mammalian blastocyst embryos of similar sizes must be a reflection of differences in the chromosomal weight of X and Y chromosomes or due to developmental differences of male and female embryos. However, while the size of embryos before blastocyst formation is highly conserved, embryos at the blastocyst stage show dynamic shifts in size. Data suggest the SGD can remain an accurate predictor of sex if the size is incorporated into the calculations.

Introduction

In Vitro Fertilization (IVF) is now 40 years old. Over that time, much has changed in the way clinics and labs treat patients and embryos, resulting in a positive IVF outcome going from a newsworthy event in the 1980s, to now accounting for about 2% of all birth occurring in the United States. However, one thing has not changed in the laboratory. We still rely heavily on an embryo's appearance (its morphology) in determining if it is transferred back into the patient or not. However, repeated studies have demonstrated that morphology cannot guarantee pregnancy (figure 1).

Therefore, IVF clinics are still searching for a better means of embryo assessment. This has led to the development of preimplantation genetic testing (PGT), an excellent means of assessing chromosomal abnormalities and identifying sex. However, numerous studies have demonstrated that simply knowing the genetics does not guarantee embryo viability. Further, PGT is expensive, time-consuming and because it requires biopsy of the embryo by laser, potentially damaging to the very embryo we hope to transfer to achieve pregnancy.
An alternative approach has been to look for noninvasive techniques. Currently, there appear to be two prevalent techniques; 1) morphometrics, which accesses both morphology and growth rates – a technique that depends on expensive specialized equipment and which has had controversial results, or 2) metabolite analysis, which again has shown somewhat spotty results.

This laboratory has previously disclosed the use of a modified specific gravity device (MSGD). The concept of the device is simple, if all other factors are held the same (embryo size, shape, and the media through which it is dropped, etc.), then the difference seen in the embryo descent time through the MSGD chamber had to be the result of changes in buoyancy which must reflect differences in embryo constituents and may reflect quality. This hypothesis was supported by data from embryos that were dropped through the chamber and returned to culture. Figure 2 demonstrates how cohorts of zygotes fell in a bell-shaped curve, with the embryos at the middle of the curve continuing to grow while those at the extremes stalled and died before reaching blastocyst. Figure 3 demonstrated a similar result in thawed blastocysts, health blastocysts continue to drop at an expected rate, while damaged embryos dropped at extremely fast rates. During this latter experiment, we made an interesting observation. Instead of a single peak at the mean, we actually had subpopulation cohorts on either side of the mean (Figure 4). This observation suggests that all other factors are equal in these two populations; the buoyancy difference might be due to differences in chromosomal weight; specifically, the sex chromosomes, where the male Y-chromosome lacks an arm. This missing arm times many hundreds of cells leading to a difference in drop-time.

However, a large study of 600 cattle embryos, where the PCR confirmation was available, only hinted that the MSGD could predict sex (see materials and methods). In that study, we recognized one of the requirements of the MSGD was not observed. Because of the time it took to measure 600 embryos (across a 2-day period), the embryos continue to develop and expand. The object of the present study was to attempt to mathematically correct for size to improve the ability it predicts embryo sex.

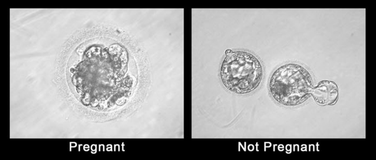

Figure 1. A comparison of embryos from an IVF laboratory demonstrating morphological quality does not always equate to pregnancy outcome.

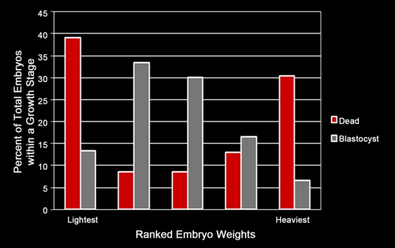

Figure 2. Correlation between embryo buoyancy and embryo development. Note that embryos on the extremes fail to develop and die while those in the center of the curve develop on to blastocyst.

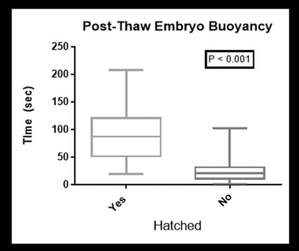

Figure 3. Correlation between embryo post-thaw survival and buoyancy. The surviving embryos, which continue to hatch, maintained buoyancy while those embryos that died during the freeze/thaw process lost all buoyancy potential.

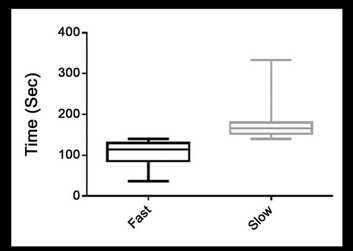

Figure 4. Observation of subpopulations on either side of the mean established of a cohort of embryos dropped through the MSGD.

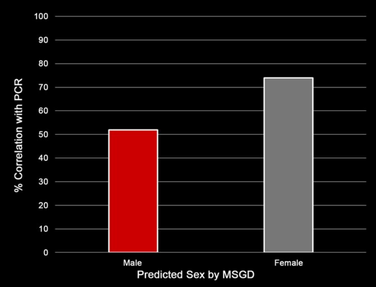

Figure 5. Original correlation of MSGD sex prediction for 600 cattle embryos and the actual sex as determined by PCR.

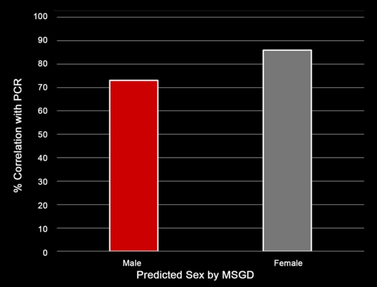

Figure 6. Recalculation of the correlation of MSGD sex prediction for 600 cattle embryos and the actual sex as determined by PCR after adjusting for changes in embryo size during development.

Materials and Methods

Previous study

1. 600 cattle blastocysts, created from in vitro matured oocytes all fertilized at the same time, were available for viability and sex determination by the MSGD
   a. 300/day across two, approximately 12 hour, days of testing.
2. Once dropped through the MSGD, embryos underwent sex determination by PCR by an outside lab.
3. Data about sex prediction was compared and analyzed (see results).
   a. While the correlation was not as high as hoped, it was noted that the embryos were developing from blastocyst, to expanding blastocyst, to expanded blastocyst across the day.

Current Study

1. As the original design of the MSGD was based on a constant embryo size, the current study attempted to correct for size using mathematics.
2. A proprietary algorithm was developed on several physical principles
3. While the algorithm was developed for actual embryo size, data here was assumed based on time of measurement and normal growth rates. Embryos were categorized as:
   a. First 100 embryos -- blastocyst (assigned average diameter 170 mm)
   b. Second 100 embryos – expanding blastocyst (assigned average diameter 300 mm)
   c. Third 100 embryos – fully expanded blastocyst (assigned average diameter 500 mm)
4. Original observations were mathematically adjusted, and the data reanalyzed

Results

1. Figure 5 demonstrates the original correlation between the MSGD and PCR. While there was a reasonable correlation for the female (73%), the male correlation was no better than a coin-toss (52%).
2. After the algorithm was applied, the correlation of the MSGD to the PCR observation was 86% for the female and 72% for the male Figure 6.
3. This represents a 14% improvement in the correlation of female prediction and a 40% improvement in the male.

Discussion

1. Data continue to suggest the MSGD might be a useful tool in assessing embryo viability and potentially embryo sex.
2. The present study was limited by the assumption of embryo size versus an accurate measurement.
3. We have just gained permission to do the first human trial of the device, where we will be able to determine embryo buoyancy but actual diameter and feed into the developed algorithm.

Acknowledgments

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