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Scientific Research

Estimation of Weight and Lipid Composition in Preimplantation Embryos from Jersey and Beef Breeds of Cattle

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Abstract

Cryopreservation is the main functional means for storage of excess embryos produced from artificial reproductive technologies; the process assumes embryos chemical nature is highly conserved across embryos of the same species. However, in practice there appears to be a high degree of variability in embryo tolerance to cryopreservation, suggesting potential differences in embryo chemistry. The objective of the current study was to develop reproducible means of estimating relative embryos weight and associating those weights to lipid content. Relative embryo weights of frozen/thawed *in-vivo* Jersey and Crossbred beef breed embryos were determined using a modified specific gravity chamber. Embryo weights were then correlated with lipid content. Results suggest that beef cattle embryos are 20% - 27% heavier than Jersey embryos ($P < 0.001$). Electron microscopy (EM) data suggest that these differences are due to divergent lipid content; Jersey embryos having approximately 36% lipid as compared to 8% in beef cattle embryos ($P < 0.01$); explaining the Jersey embryos lighter weight.

Keywords

Estimated Weight, Embryo, Cryopreservation, Cattle

1. Introduction

The first successful cryopreservation of a preimplantation embryo was performed in 1972 when the embryo was cooled to −80˚C at 1 degree/minute and then submerged in liquid nitrogen [1]. Cryopreservation remains the

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main means of storing embryos and has become standard practice in reproductive industries, primarily human and bovine. While cattle breeds such as, Angus, Simmental, Herefords and others survive current cryopreservation techniques with conception rates approaching 60% to 70%, cryopreserved Jersey cattle embryos produce conceptions at a rate of approximately 20%, very similar to that of the human species [2] [3].

At the whole body level, the main defining characteristic of Jersey cattle is their genetic predisposition to produce more butterfat than other bovine breeds, indicating that their cellular biochemical makeup includes more lipid content than other bovine species [4]-[7]. It has been suggested that the low conception rates in Jersey cattle are associated with embryo intracellular lipids, which have been linked to cell damage during cryopreservation [1] [4] [8] [9]. Presence of intracellular lipids in bovine oocytes and embryos has been shown to account, at least partially, for the low post thaw recovery rate [4] [9]-[11]. However, these differences in embryo lipid content have not been quantified between different bovine breeds or across species [2] [11]-[13]. Access to this quantification of lipid content could alter the way that cryopreservation methods are applied [4] [12]-[15]; particularly with mammals that possess cells with known higher lipid content (*i.e.* humans with higher Body Mass Index) [15]. Determination of specific gravity has been a common way to estimate lipid content in live individuals [16] [17] and foodstuffs [18]. Using this method as a format the current study aims to develop a reproducible means of estimating relative embryos weight and then relating those weights to determine lipid content. Alteration of the cryopreservation technique could allow for greater success rates in both freezing and thawing of high-lipid species or certain breeds within that species.

The objective of the current study was to develop reproducible means of estimating relative embryos weight and then relate those weights to lipid content. The cryopreservation process assumes that water is highly conserved across embryos of the same species. Therefore, any dramatic shift in the embryos' chemical nature might lead to failure of the cryopreservation process. Using techniques developed in this laboratory, embryo estimated weight and lipid content were compared between Jersey cattle and beef cattle breeds.

2. Materials and Methods

All embryos used in the experiments were *in-vivo*, day-six compacted blastocysts and were donated from commercial sources. The beef cattle embryos were obtained from the Trans-Ova Genetics Satellite Center (Abernathy, TX). The Jersey cattle embryos were obtained from the US Jersey, American Jersey Association (Reynoldsburg, OH). Frozen *in-vivo* embryos were utilized due to the limitations of the Jersey cattle industry obtaining fresh embryos, which were very much in demand during the experiment. All embryos had previously been frozen using traditional slow freeze protocols using ethylene glycol, a traditional cryoprotectant. Embryos were then thawed according to industry standards. Fifty Jersey and 50 beef cattle embryos were transferred one at a time to the experimental specific gravity apparatus.

2.1. Specific Gravity

As previously stated, specific gravity has been a common way to estimate lipid content in live individuals [16] [17] and foodstuffs [18]. A modified specific gravity chamber was constructed that allowed the visualization of embryos and a standard bead which was embryo shaped and of known weight, as they "dropped" a predetermined distance. The chamber was constructed of a 0.5 mL semen straw (Cryo Bio System; Paris, France). A distance of 1.00 cm was marked on the straw. Prior to experimentation, the straw was rinsed with human serum albumin (HSA) (Irvine Scientific, Santa Ana, CA), to coat the inside of the straw, to prevent embryos from "sticking" or being drawn by electrical charge to the sides of the straw. Following coating with HSA, the straw was filled with one of two medias of known specific gravity: 1) P-1 holding medium, or 2) F-2 freezing medium (Irvine Scientific, Santa Ana, CA). These mediums were chosen to prevent the media itself from potentially damaging the embryo during the process, which would allow utilization for future breeding purposes if desired. Additionally, two mediums were used in order to verify the results ensuring that the specific gravity differences seen were true variation between breed types.

The straw containing media was attached vertically to a standard ring stand and illuminated. The drop region of the straw was visualized with a Nikon SMZ2 (Nikon Inc., New York, NY) attached to an articulated arm boom microscope stand (American Optical Scientific Industries, Southbridge, MA). Embryos and standards were released at the top of the straw and the descent was timed using a stopwatch and recorded out to the tenth of one second for all procedures. Care was taken to "place" the embryo on top of the column without inducing

any downward movement. Resulting times were then entered into the appropriate equation to estimate the embryos' weight. This process was repeated using 50 additional embryos of each breed in both the P-1 and F-2 media and the resulting data entered into that equation to confirm the approximate weights.

2.2. Lipid Quantification

Ten embryos of each breed were used (n = 20) were used in the EM studies. A small amount of Acidic Tyrode's Solution (Irvine Scientific, Santa Ana, CA) was added and allowed to sit until most of the zonapellucida was removed enzymatically. Then HSA was added in equal amounts to that of the P-1 and Tyrode's to stop the enzymatic degradation process. The embryo was then transferred to a gluteraldehyde solution, 3% paraforinaldehyde and 1.5% gluteraldehyde. The embryos were held in the gluteraldehyde for a minimum of 24 hours to ensure complete fixation. Following fixation, embryos were then transferred to the Electron Microscopy Center at the Texas Tech University Health Sciences Center (Lubbock, TX) where they were further processed for transmission electron microscopy using standard techniques. Random sections of each embryo were observed and photographed on a Hitachi H-7650 TEM by a trained microscopist.

All resulting EM work was placed on a computerized grid from Adobe Photoshop CS2 (Adobe Acrobat, San Jose, CA). The grid places 2 × 2 cm^2 uniformly over the digital image. The squares were then qualified as either lipid containing or lipid non-containing by a single observer. A square containing more than 50% lipid was scored as lipid containing. In order to be included in the total value counted in the lipid quantification a square had to be at least 50% occupied by a cellular image. To calculate lipid content in the embryo, the lipid-containing squares were divided by total squares and multiplied by 100 to give a percentage of lipid found in the embryo section. All EM images were counted and then matched so that only those with corresponding magnifications in the opposite breed were included in the results. If multiple images at a given magnification were present, the ones with the greatest lipid content percentage present were used.

3. Statistical Analysis

Following data collection, descriptive statistics were performed for each set of data and an intra-assay CV established between the various standard curves to determine precision. Breed differences in lipid content were determined using student's T-test. All statistical comparisons were made using the Statistical Package for the Social Sciences (SPSS ver 12: Chicago, IL).

4. Results

As expected, the relationship between weight (density) and time of descent was not linear but curvilinear on a logarithmic scale (see **Figure 1**).

Using the two points established by the bead type; borosilicate (BS) or barium titanate (BT), points for zero time and zero weight were extrapolated mathematically resulting in four similar equations for the slope of the curves:

BS/P-1; ($y = -0.40\ln(x) + 2.070$, $R^2 = 0.987$);
BS/F-2; ($y = -0.40\ln(x) + 2.449$, $R^2 = 0.986$);
BT/P-1; ($y = -0.27\ln(x) + 1.353$, $R^2 = 0.993$);
BT/F-2; ($y = -0.22\ln(x) + 1.494$, $R^2 = 0.983$).

Mathematical comparison of the results indicated the four curves established using the borosilicate (BS) or barium titanate (BT); were within 7% of each other and the <±11% of the mean weight supplied by the manufacturer. Further, while there was observable variation in the zonapellucida thickness between embryos, the variation in the time of descent between embryos within the breeds appeared normally distributed around the mean and negligible in accounting for the variation observed between the breeds (42.4 + 1.1 s vs. 58.2 + 2.3 s in P-1 media and 173.6 + 4.5 s vs. 214.5 + 8.8 s in F-2 media for beef vs. Jersey embryos respectively ± S.E.M.).

Using embryodescent times and the previously established standard curves, estimated weights were established for the embryos of between 0.2 and 0.5 μg. The average weight for beef cattle embryos being 0.40 ± 0.11 μg/mL ($P < 0.001$) and Jersey breeds 0.32 ± 0.11 μg/mL ($P < 0.001$) (**Figure 2**); to the authors' knowledge the first true estimation of embryo weight. Demonstrating Jersey cattle embryos being, on average, 21.3% times lighter than beef embryos at the same stage of development.

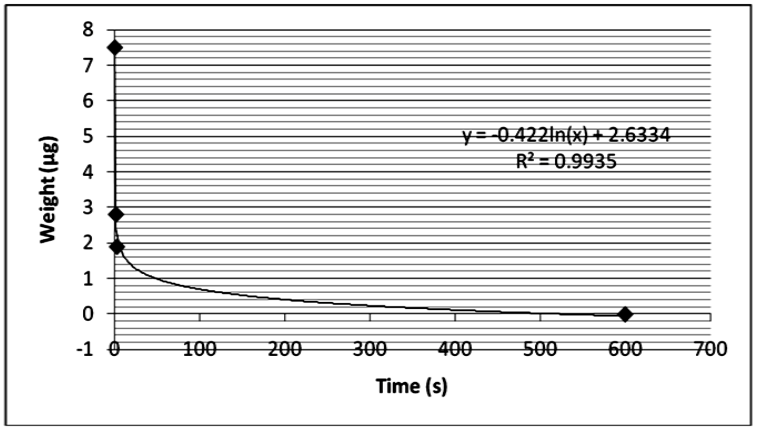

Figure 1. Representative curve of curvilinear descent of standardized beads creating a mathematically extrapolated zero time and zero weight points for use in creation of a standard curve to determine embryo weights through use of a unique specific gravity technique.

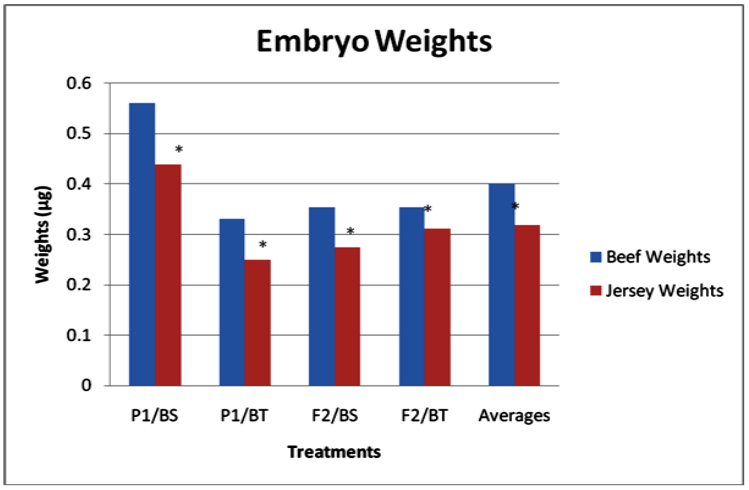

Figure 2. Determination of breed differences in embryo weight ($P < 0.001$) between Jersey and beef cattle breeds as determined by a unique modified specific gravity technique.

The electron microscopy data suggest that Jersey cattle embryos (55.2% ± 22.0%) contained significantly more lipid; ($P < 0.001$) than beef cattle embryos at the sample stage of development (6.0% ± 6.8%) (see **Figure 3**).

5. Conclusions

Low pregnancy rates are suggested to be associated with intracellular lipids of the embryo, which have been linked to cell damage during cryopreservation [1] [8]. These intracellular lipids in bovine oocytes and embryos have been shown to account, at least partially, for the low post thaw success rate in both *in-vivo* and *in-vitro* derived embryos [10] [19]; however, embryo lipid content has not yet been quantified.

Data from this study suggests that there is considerably more lipid content found in Jersey breed embryos. Differences can be seen both in the weight through a modified specific gravity technique, comparing Jersey to beef cattle breeds, and in the lipid quantification through electron microscopy allowing for the same breed comparisons. From the statistical analysis, it is evident that the Jersey embryos are considerably lighter in weight than that of the beef cattle breeds. Additionally, the statistical analysis shows that there is a considerable amount of more lipids present in Jersey cattle when quantified. Because of this, it is reasonable to infer that the lighter

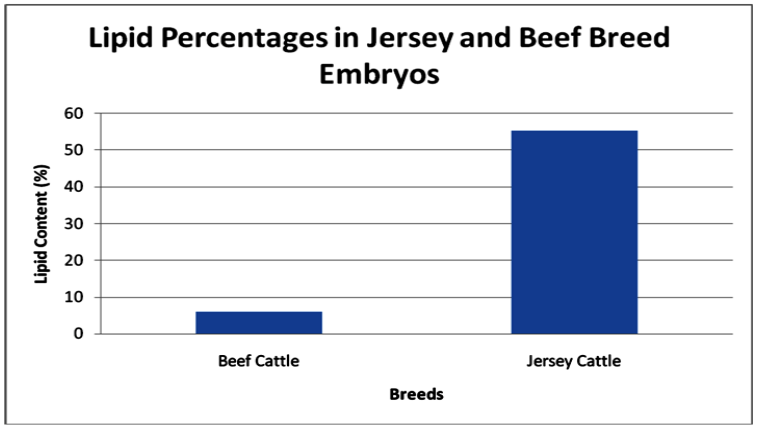

Figure 3. Determination of breed differences in embryo lipid content ($P < 0.001$) between Jersey and beef cattle breeds as determined by quantification of lipid content in random electron micrographs.

weight seen in the modified specific gravity technique is due solely to the excess lipid seen in the Jersey embryos.

Ongoing research in this laboratory is being conducted to compare fresh *in-vivo* embryos and those from various other models along with varying stages of cellular development.

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