final report

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Prepared by: Jeremy Thompson
The University of Adelaide

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Quantitative image assessment of embryos to predict pregnancy in embryo transfer programs

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Abstract

Female-focussed reproductive technologies have modest adoption in the Australian cattle industry. In part, this is caused by low and /or variable pregnancy rates. Here we assessed the predictive capacity for pregnancy establishment from microscopic imaging of routinely selected transferable cattle embryos prior to transfer. This incorporated analysis of features built from custom-algorithms. A total of 476 multiple ovulation and embryo transfer (MOET) embryos (266 Pregnant [P] and 100 Non pregnant [NP]) at Day 7 and juvenile in vitro fertilised embryo transfer (JIVET) embryos (same stage: 62 P, 63 NP) were imaged and analysed. Mid-section and bottom images of embryos were collected under white, or red, blue and green filtered differential interference microscopy. Images were corrected to ensure consistency between different days of collection. Supervised analysis specifically targeting P/NP outcomes were conducted. At present, the probability of an accurate prediction for MOET embryos is 88% and for JIVET embryos is 96%. A probability over 95% is considered a highly predictive assay. For images from JIVET embryos, a cluster in an unsupervised analysis was highly predictive of pregnancy failure, suggesting that there are common features in some JIVET embryos that will not form a pregnancy. Cross validation and further images taken across breeds, management and regions is now required. With further data, we are confident that an accurate image-based assessment for pregnancy for each embryo is achievable. Direct value to breeders is estimated at an additional $30,000 for every 1000 embryos transferred with a modest 5% pregnancy rate improvement.

Executive summary

Objective
To date, uptake of female focused advanced artificial reproduction (ART) in bull breeding herds remains low which limits the rate of genetic gain achievable. The aim of this project was to improve efficiency and thus reduce costs per calf making advanced breeding methods more attractive. This is expected to lead to significantly greater adoption of female ART in bull breeding herds. The major interlinked limitations to the use of embryo transfer technologies by bull breeders stem from the modest but variable pregnancy success rate of transferred embryos which for Multiple Ovulation and Embryo Transfer (MOET) currently averages approximately 60% and for In vitro Fertilization (IVF) technology, pregnancy rates are in the order of 40% for mature cows and even poorer and very variable for juvenile animals (JIVET). This low success rate means a large numbers of recipient cows need to be managed, and hence there is a significant on-farm logistics challenge and high overall cost per live calf born.

Within ART programs, it is the experience of the embryologist to discern the quality of an embryo that governs if the MOET-recovered or IVF-derived embryo is chosen for transfer or not. The current method recommended by the International Embryo Technology Society (IETS) grades embryos based on morphological classification as 1. Excellent or Good, 2. Regular, 3. Poor. This embryo grading approach is subjective and operator dependent. Embryos classified as 1, (excellent or good) have higher successful pregnancy outcomes than regular embryos. However, there remains considerable scope for improvement in the assessment (Rocha *et al.* 2017). Improving objective assessment of embryos by analysis of digital images is an ideal approach as it is non-invasive, objective, with high reproducibility, and can be carried out in "real time" thus facilitating the selection of freshly produced embryos for immediate transfer, or freezing. The ambition is to increase embryo transfer success rates by 10% on average from the same number of embryo transfers. We believe this is achievable given the preliminary data from application of the technique in predicting in human embryo transfer success.

The University of Adelaide was the lead research partner. Holbrook Vet Centre, Holbrook NSW provided the embryo production, image collection and pregnancy diagnosis. Quantitative Pty Ltd. undertook the image analysis.

Methodology
High resolution images of day 7 embryos were collected on properties across southern Australia from a range of breed types including Angus, Poll Hereford, Wagyu and Composite cattle. Images were collected on embryos from October 2017 to March 2018 with day 60 pregnancy subsequently supplied. We applied a novel digital imaging technology, including grey-level co-occurrence matrices (GLCM) image analysis, plus a set of custom-fitted features (algorithms, proprietary to Quantitative) on individual cattle embryos that were adjudged suitable for transfer by IETS criteria. Such technology is being developed for the human fertility field to increase embryo transfer success rates. We had also assessed our approach experimentally comparing two sources of embryos that by standard microscopy were not discernibly different in morphology. This demonstrated that the digital analysis approach taken here was able to segregate these two different sources of embryos, where other techniques were not (Sutton-McDowall *et al.* 2017).

Adjustments to the normal operating work flow for ET was required to accommodate the imaging of embryos. Novel 'slide-holding' 3-D printed devices were created to enable ease of operations, which prevented the risk of valuable embryos being lost by mishap. Nevertheless, the time from recovery of embryos from a uterine flushing into ET straws was lengthened for each flush which prolonged operations. This meant not all embryos that were potentially available were imaged.

Following the ET program, a total of 491 embryos were imaged; MOET embryos (100 Not-pregnant, NP and 266 Pregnant, P) and JIVET (63 NP and 62 P). Fifteen embryos were removed using an outlier filter leaving 476 analysed.

Digital image data was conditioned to remove irregularities such as background light changes over time and reflection from different sources on different days of embryo collection.

Results

Initially, a GLCM analysis using the best 10 features (algorithms) was applied to the white light illuminated mid-section images of all embryos, which when plotted as a Receiver Operator Curve (ROC, a plot of true positive and false positive predictions) delivered an area under the curve of 71% accuracy of prediction, which is entirely in agreement with existing GLCM human embryo data. However, this was greatly improved (ROC = 86%) by applying the best fitted 10 Quantitative custom-designed features in a supervised analysis; i.e. deliberately attempting to link the pregnancy outcomes with a set of features.

A deeper analysis using all available images that included the mid-section and bottom images, inclusive of white, red, green and blue filtered images was undertaken. This was further segmented into the MOET or JIVET embryos. This improved accuracy of prediction with a supervised analysis for MOET embryos (88.5%) and JIVET embryos (96%), the latter meeting the criteria of a predictive assay.

In addition, an unsupervised analysis of the JIVET embryo images revealed a distinct cluster of 12/13 NP observations. This strongly suggests that there are features within the JIVET embryo that can be recognized and highly predictive of failed pregnancy but were not discernible by visual (microscope) inspection to experienced field technicians. It is likely that this cluster significantly contributed to the ROC result. This outcome is extremely encouraging in that it demonstrates the potential to objectively assess JIVET embryos.

Cross-validation work is required, as this will generally reduce the predictive power of a set of features by approximately 10%. This should include increasing the data set by increasing other sources of embryos (different breeds and breed types (e.g. *indicus*), production regions, nutritional state of donor and recipient) and also by adding more predictive features. The larger the data set, the more predictive it will become.

We estimate that with further data collection and model development it is feasible to achieve a 10% increase in pregnancy rates for MOET and IVF. Such a result would significantly reduce costs per calf born and make MOET and IVF a more viable option for bull breeders across Australia. Moreover, the same technique can potentially be applied to sperm analysis (for artificial insemination) and in sheep.

Conclusion

This proof of concept project has successfully demonstrated the utility of analysis of high resolution images in identifying the most suitable embryos to transfer to recipients. The outcomes described here is consistent with the aim of the study, to develop technology that will improve the efficiency of embryo transfer by maximising pregnancy establishment and minimising the number of non-pregnant recipients. Our modelling reveals that in a 100 cow recipient herd, for every 5% increase in MOET pregnancy rate (calf weaning rate), e.g. from 50% to 55%, there is a $3000 increase in income for contract breeding. Moreover, increasing pregnancy rate and reliability is expected to increase demand for female ART thus leading to increased rates of genetic gain.

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1 Background

1.1 The application of Advanced Reproductive Technologies

Delivering rapid genetic improvement to Australian beef herds depends on a female-focussed advanced reproductive technology (ART) program. Rapid improvements can only be achieved by capturing both superior cow and bull genetics simultaneously and transferring high numbers of their embryos into as many recipients as available. This is achievable with only two technologies: Multiple Ovulation and Embryo Transfer (MOET) or the In Vitro Fertilisation (IVF) embryo. Juvenile IVF and ET (JIVET) is a further form of IVF, where cows that are 6-months or younger are programmed for oocyte collection. MOET has the advantages of delivering higher quality embryos, but is limited in the number of embryos that can be produced from a single cow in any one calendar year. IVF embryos represent a more flexible option for beef breeding, but has been limited in the past by significantly lower pregnancy rates, especially for JIVET, relative to MOET embryos.
Pregnancy failure following mating, AI or embryo transfer is a major cost both in lost genetic improvement and financial costs. Within ART programs used for breeding from elite genetics, minimising failed pregnancy establishment in recipient herds is critical to recipient management, as a failed pregnancy in a recipient cow has management implications and an economic cost. Yet maximising the calf production from all oocytes and embryos recovered from an elite donor is essential to maximise the genetic improvement opportunity.

Assessing the quality of an embryo immediately prior to transfer is the only opportunity for MOET-derived embryos, as they are recovered and either cryopreserved or transferred within hours of collection. For IVF embryos, there is more scope to examine the quality of the growing embryo during development in the laboratory. Nevertheless, the most informative stage of development concerning the quality of an embryo is immediately prior to transfer.

1.2 1.2 Imaging of embryos to determine their quality

Imaging of embryos under microscopy to assess their quality is normally performed by experienced embryologists. It is a skill to judge if an embryo is either compromised so likely not to form a pregnancy, or appears morphologically normal and is likely to form a viable pregnancy. There are guides of classifications of the morphology which assist in selection, for example IETS Manual, and (Bo and Mapletoft 2013). Nevertheless variability between embryologists remains and what is required is an objective assessment system (Rocha *et al.* 2017).

Imaging of embryos to capture quantitative data is not a new approach (Lim et al 1999). However, with the advent of improved imaging capacity (high resolution cameras) and algorithm development designed to describe the relationship of pixels to each other in an image (e.g. machine learning and neural networks), coupled to the increasingly faster computational speeds, there are publications emerging that utilize these tools to describe embryo stage and quality, and are making comparisons to operator morphological grading (e.g. (Melo *et al.* 2014; Rocha *et al.* 2017)

2 Project objectives

1. Test of the accuracy with which the algorithms developed from GLCM analysis and of custom-features analysis of day 7 embryo images can predict pregnancy rates from transferred embryos
2. Assessment of the feasibility and practicality of capturing appropriate images for analysis in the field in a commercial setting

3. Quantification of potential cost savings (per live calf born) and logistics benefits of objective assessment of embryos prior to transfer

2.1 Final Report Milestones

1. Signing of agreement
2. Report describing that imaging of 500 embryos is complete
3. Report describing initial image analysis including prospective predictive capacity
4. Accuracy with which GLCM analysis can predict resultant pregnancy outcomes
   Optimising of logistics and protocols for capturing appropriate images for analysis in commercial settings
   Quantification of potential cost savings (per live calf born) and logistics benefits of objective assessment of embryos prior to transfer
   Recommendation for further work

3 Methodology

3.1 The microscope system used

The microscope system constructed for this application consisted of an Olympus IX73 inverted microscope with contrast enhancement, specifically, differential interference contrast (DIC) capability. The excitation source was a 100W halogen white light source and three wide band colour filters, blue, green and red all setup to be interchangeable in the excitation path. This potentially allows investigation of 12 colour channels, white and three colours in excitation and three emission channel (Red-Green-Blue).

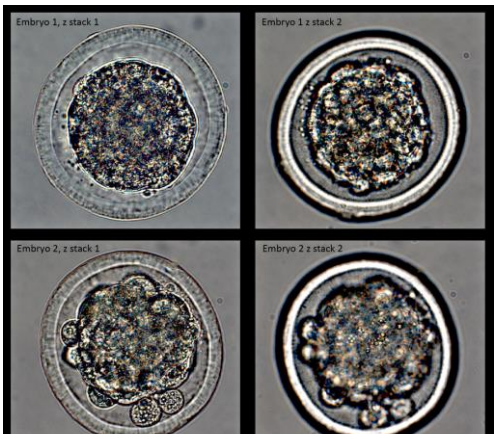
Figure 1. The microscope and camera employed (top row). The vets at Holbrook collecting the embryo image data prior to transfer (bottom row).

The objective chosen was a UPLFLN40X air/dry with a numerical aperture of 0.75 and 0.51mm working distance being an ideal choice for general field instrumentation. The camera chosen was a USB XIMEA colour CMOS with 2464x2056 pixel resolution and greater than 40dB signal to noise ratio. A colour camera was chosen to allow us to extract subtle colour features that might correlate to embryo quality.

3.2 Imaging/data collection

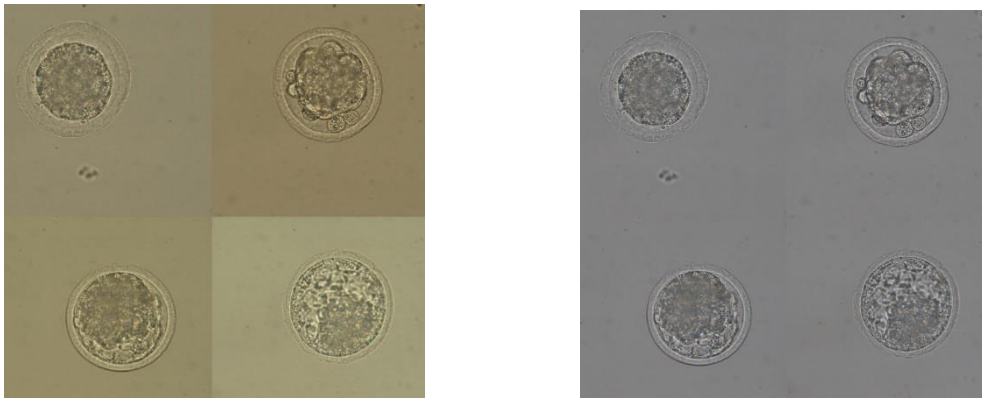
The data was collected from properties across southern Australia. As such, the environmental background light changes with background, time of day, operator clothing, etc. These conditions need to be tolerated as normal field conditions. An imaging protocol was developed in close association with the HVC staff and practised, to readily locate the embryos within the specialised embryo holding dish and novel 3-D dish support device. Imaging was performed separately under the four illumination conditions (white light only then red, blue and green filters). The embryo was imaged at the bottom of the dish and then a mid-section, where the Zona Pellucida (the clear coat around the embryo) was clearly in focus. A total of 491 embryos were imaged; MOET embryos (100 Not-pregnant and 266 Pregnant) and JIVET (63 NP and 62 P). Fifteen embryos were removed using an outlier filter leaving 476 analysed.

Figure 2. Two different looking compact morula embryos imaged using white light at the two z positions.



All images were carefully 'conditioned' and filtered from the electronics "noise" produced by the USB ports of the XIMEA colour camera without corrupting any information. The other data conditioning step required is a standardisation to correct for slight differences in ambient light conditions during imaging. This includes "white balancing", where colour correction is standardised based on the background colour bringing all embryo images to have the same background allowing us to investigate subtle colour differences between embryos independent of differences in ambient lighting (Figure 3).

Figure 3. (Left) Raw images (white light illumination only) of four randomly chosen embryos showing the variance in ambient illumination and colour, (Right) we see same images standardised for colour and intensity (now the only colour variation is the subtle embryo pixel colour differences).



Visually, the colour information within these embryo images is very subtle, in fact the white balanced images (Figure 3) appear simply grey intensity images. However, this is not the case. The RGB colour channels are very highly correlated which makes the images appear grey to human vision, but we were able to apply mathematical transforms to de-correlate the data and colour

enhance the images that highlighting very subtle differences and features in the original colour image information (Figure 4).

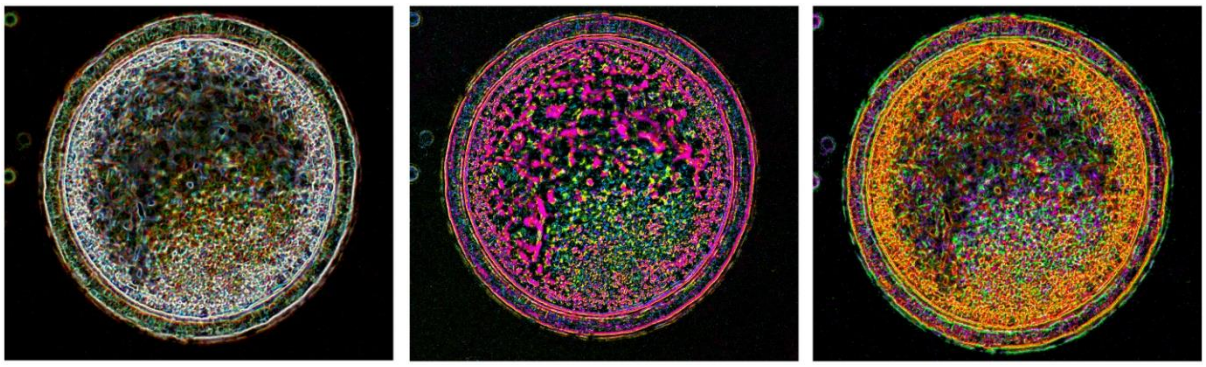


Figure 4. Examples of colour feature images (same embryo but not same as in figure 1), here we have used a super-resolution reconstruction method to capture some very detailed colour information.

3.3 Analysis

The next phase was to develop algorithms to distinguish distinct gross morphological features of any given embryo image (such as zona pellucida, inner cell mass, blastocoel cavity and the distinction between morula and blastocyst stage embryos). Additionally, we developed a further algorithm to first recognize the embryo then extract the whole embryo from each of the images. Such extraction and cropping is necessary for the analytical algorithms and greatly reduces computation time. The auto crop algorithm worked well, with a 90% success rate, with all remaining embryos being cropped manually, which was a tedious process.

3.4 Additional feature extractions

Embryos images are an extremely rich source of features and patterns, and they present potential for the calculation of an enormous suite of features. Therefore, apart from undertaking a grey level co-occurrence matrix features analysis, we also generated a suite of over 40,000 custom features (algorithms) capturing textural, morphological and colour patterns. Examples of such feature images are shown in Figure 5, where the pattern of shading is a measure of a particular feature value.

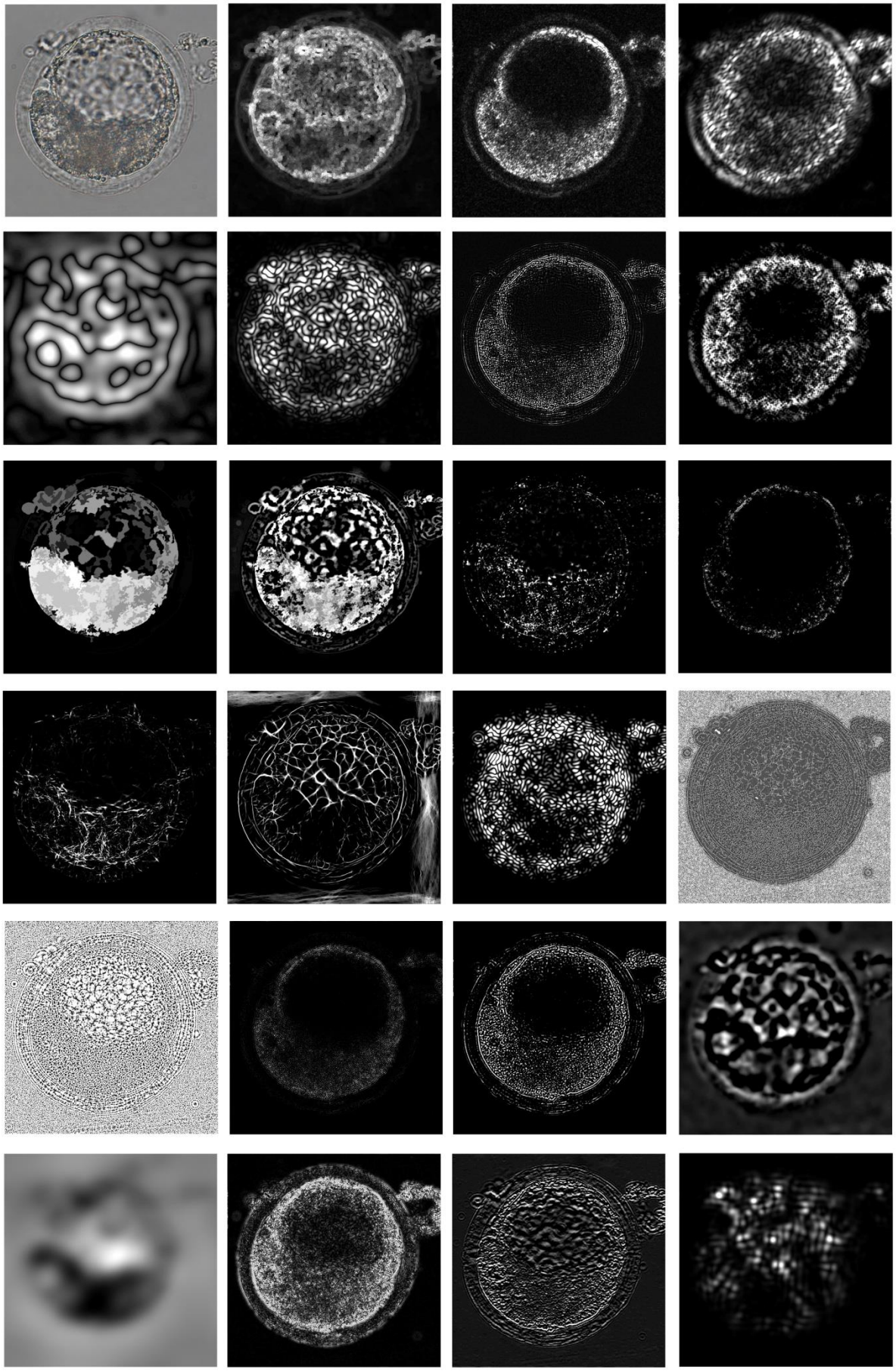


Figure 5. Examples of feature images (same embryo), highlighting the diversity of information.

4 Results

4.1 Preliminary results – Unsupervised analysis

An initial assessment was performed on a partial subset of MOET embryos. From the ~40,000 features we selected a smaller best subset of approximately 1,500 features that met a set of cut-off criteria. These 1500 features were then used to build an unsupervised model based on the ten best features that maximally clustered the data into three clusters (Figure 6). To visualise these clusters on a two dimensional plot we 'project' the data from these ten features into a 2D space using a mathematical algorithm that maximises the spread of the data across the 2D space, because it is impossible to visualise the clusters in a 10 dimensional space. Each dot represents where the features of an image of an individual embryo fits within the projected model in relation to each cluster created by the model.

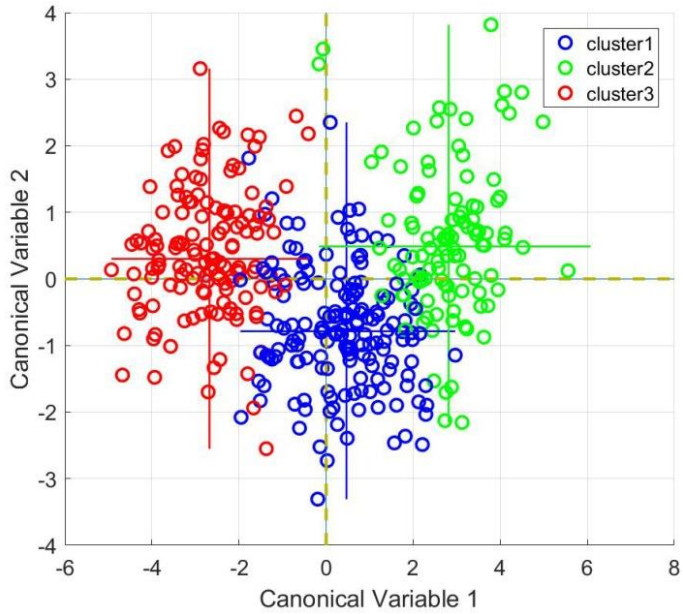


Figure 6. Unsupervised analysis using informative embryo features finds a distinct naturally clustering of the embryos. P value < 10e-40 for all pairwise group tests.

We then matched these clusters to outcomes such as pregnancy establishment, stage of development, embryo quality assessment (IETS criteria), etc. Although the distributions of these clusters were of some interest, no particular cluster was particularly overrepresented with any outcome measure, with the greatest association being with stage of embryo development, a somewhat predictable outcome.

4.2 Preliminary results – Supervised analysis using only GLCM features

We then fitted a best discriminating model using only grey level co-occurrence matrix (GLCM) features (the best 10), as was originally specified for this study, then by way of comparison to a best model built using a set of ten features chosen from any of the 40,000 additional Quantitative custom features. Once again to visualise the discrimination of the data we 'project' the data from these ten features optimally into a 2D space using a mathematical algorithm that maximises the separation

between the two labelled group (data not shown. The performance of this model is captured in a receiver operator's classification (ROC) curve, which has an area under the curve value of 1 for perfect classification. This resulted in a prediction capability of 71%, which is in line with most GLCM approaches (Figure 7).

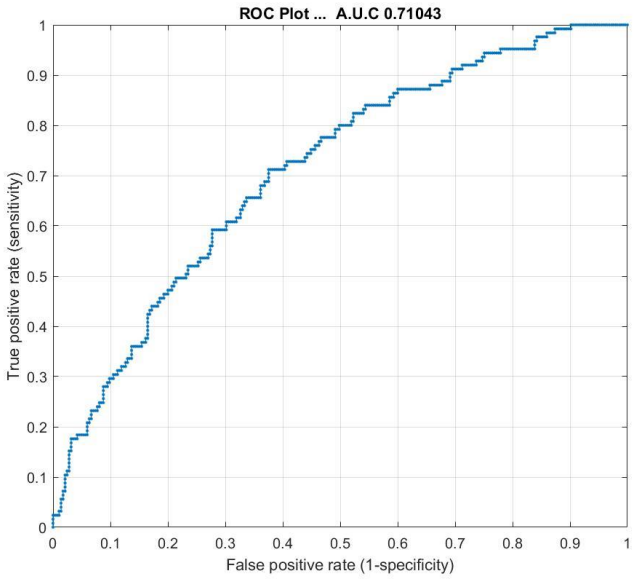


Figure 7. Receiver operator curve ROC shows an area under the curve value of ~71% for a discriminating model using ten best GLCM features.

4.3 Supervised analysis using Quantitative custom features

To compare against the GLCM approach, we applied the best 10 of the 40,000 additional Quantitative custom features. This improved the prediction capability significant (86%) (Figure 8).

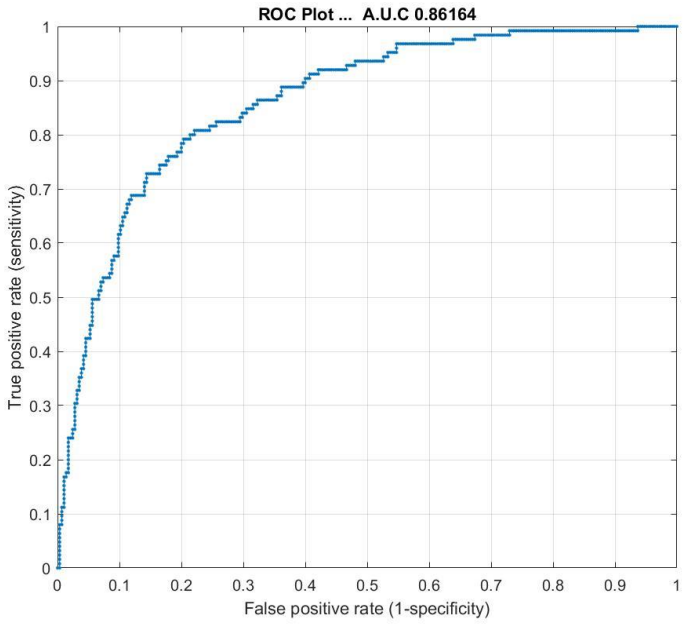


Figure 8. Receiver operator curve ROC shows an area under the curve value of ~86% for a discriminating model using the best 10 Quantitative features.

4.4 Complete analysis – Unsupervised analysis of all embryos

Firstly, we present all data (476 embryo) using a model built by selection from an initial pack of 40,000 features taken with white light and three excitation colours (W, R, G, B) and two image planes, midsection and bottom, amounting to a total of 320,000 features (Figure 9).

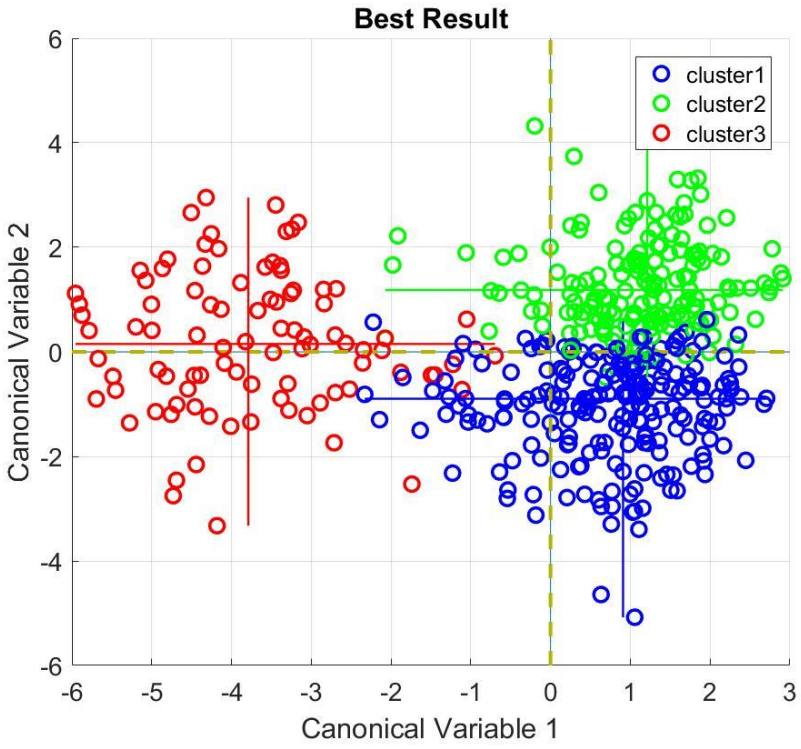


Figure 9. Unsupervised model building process selected 10 most informative embryo image features and finds a distinct naturally clustering of the embryos, now with significantly more separation than previously. P value < 10e-40 for all pair wise group tests.

On characterisation of outcomes, Cluster 1 and 2 were predominantly MOET embryos and Cluster 3 was predominantly JIVET embryos. Furthermore, where Cluster 1 and 2 were equally overrepresented with pregnancy success, Cluster 3 was equally represented with P and NP results. This led to a further investigation of just the JIVET embryos.

4.5 Unsupervised analysis of JIVET embryos.

Because of the limited data set, only 5 features were best fitted into 3 clusters in an unsupervised analysis of the JIVET embryos. Nevertheless, this revealed 3 separate clusters (Figure 10). Of significance was the proportion of embryos in Cluster 3 that represented predominantly NP recipients (12/13), suggesting that for these embryos, even 5 features can account for 20% of the embryos that will not form pregnancies (Figure 11). This was independent of whether these embryos were transferred as 'fresh' or cryopreserved (vitrified) and then thawed.

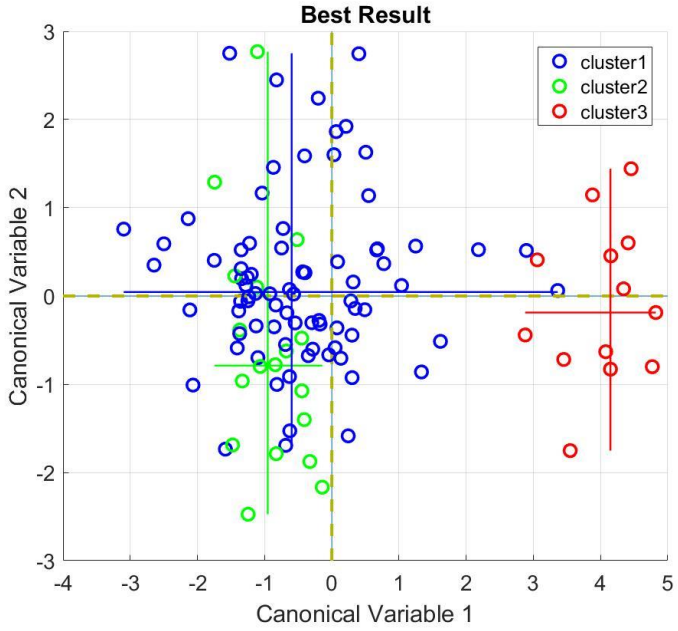


Figure 10. Unsupervised model building process selected 5 (being restricted by the limited amount of data) most informative IVF embryo image features and finds a distinct naturally clustering of the embryos (Pval between cluster 3 and others <10-6, between 1 and 2 <0.00045)

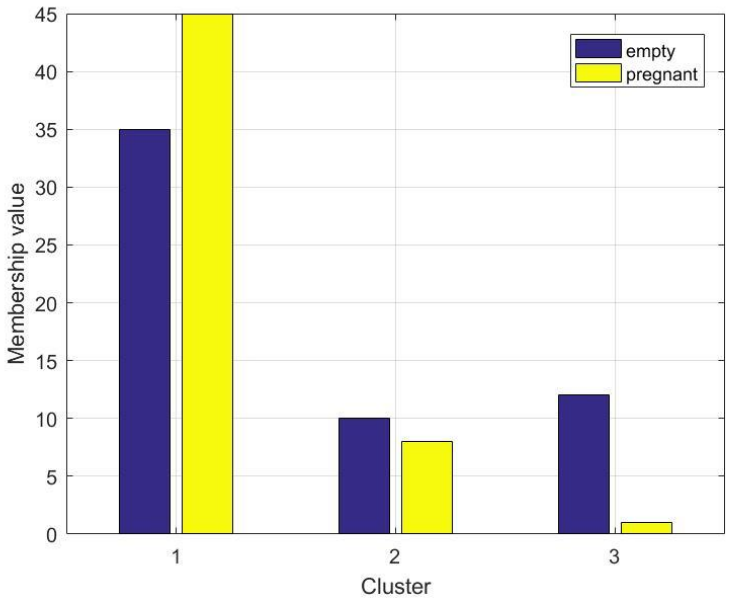


Figure 11. Cluster analysis for NP or P outcomes reveals that Cluster 3 is associated almost exclusively with NP recipients, but does not capture all NP embryos, suggesting it is sensitive to some other variable.

In contrast, the unsupervised analysis of MOET embryos did not associate with any particular outcome measure (data not shown).

4.6 Supervised model for P and NP in embryos

A best discriminating model was built using only a small selection of 12 features, being the best working combination found, with the total number of features being constrained based on the size of the data set (see Section 4.7). This model uses all the images available.

Projecting the data from these twelve chosen features into the 2D space revealed an ROC curve of 88.5% (Figure 12).

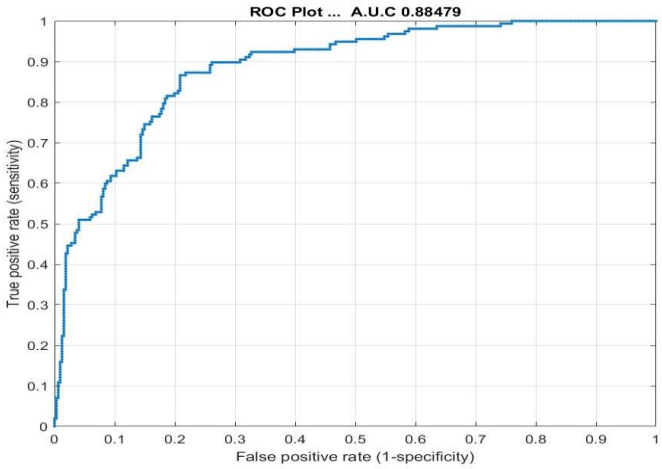


Figure 12. Receiver operator curve ROC shows an area under the curve value of ~88.5% for a discriminating model build using all the data points and the best selected twelve Quantitative custom features.

4.6.1 Supervised analysis for JIVET embryos only

We repeated the exercise for JIVET only embryos using a model based on seven features being restricted by the amount of data available (Figure 13). The model for the IVF appears to perform significantly better than one built using MOET and IVF, showing an ROC score of ~96% (Figure 14).

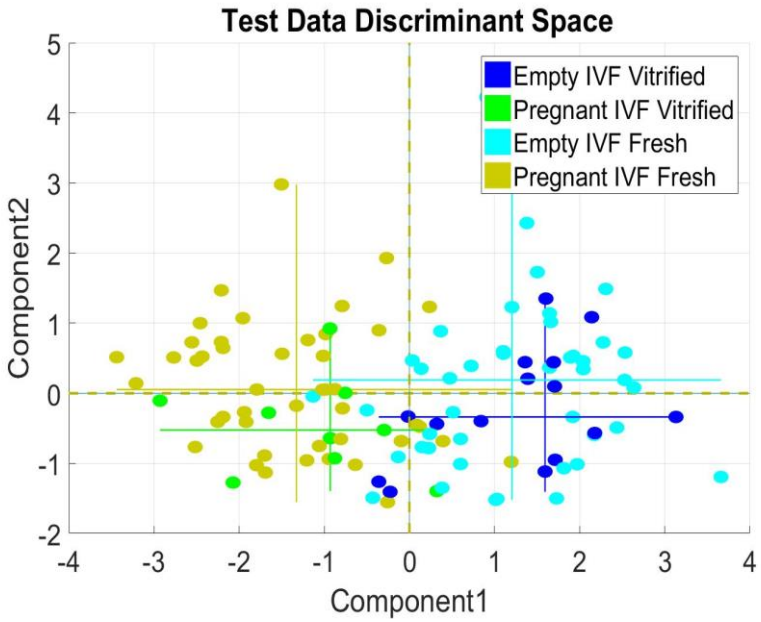


Figure 13. A two dimensional plot visualising the discrimination of the model for NP vs. P recipients, using all IVF data only, using the best selected twelve Quantitative custom features.

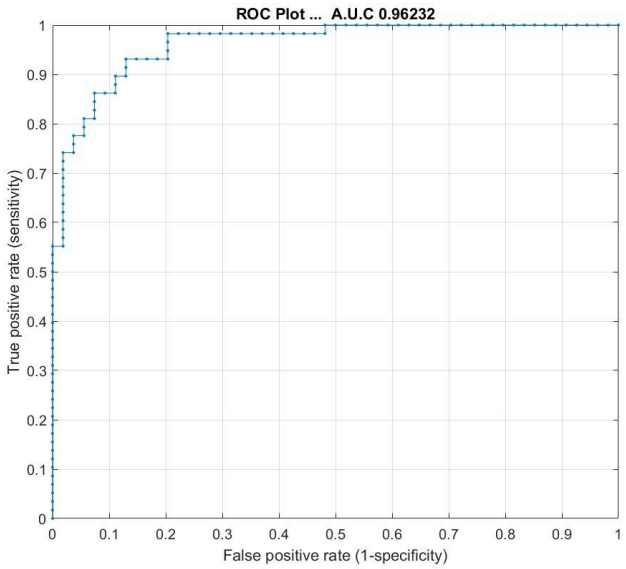


Figure 14. Receiver operator curve ROC shows an area under the curve value of ~96% for a discriminating model build using all the IVF data points and the best selected seven Quantitative custom features.

4.6.2 Supervised analysis of MOET embryos only

We repeated the exercise for MOET only embryos using a model based on ten features being restricted by the amount of data available. The model for the MOET embryo appears to perform slightly worse than one built using MOET and IVF (showing an ROC score of ~87.9%) (Data not shown).

5 Discussion

5.1 Advancing the field of embryo predictive pregnancy establishment

Our evaluation of the literature that is available at present time reveals that we have progressed further with computational imaging analysis for pregnancy prediction following embryo transfer in cattle than any other study. Nevertheless, recent publications, e.g. (Rocha *et al.* 2017), demonstrate there is considerable interest in this area, both in livestock production and human infertility research. Our point of difference from other studies is the use of Quantitative's custom "features" algorithms, and the establishment of a specific microscope set-up, with filter set and high pixel density camera. Most other groups will most likely use a less specific microscopy set-up, and will most likely pursue either a GLCM analysis approach (with publicly available algorithms) or a "Deep learning – neural network" approach. It is likely that the latter will produce predictive algorithms, but the amount of data required to generated and validate a neural-network generated model will be much greater than the approach taken here (Gosnell, personal communication).

A GLCM analysis approach was originally promoted for this study, but in the intervening period from grant submission to initiation of analysis, it was clear from other studies conducted by us, e.g {Sutton-McDowall, 2017 #7246; Gosnell and Ryan, unpublished data), the use of Quantitative's custom 40,000 algorithm resource will provide a more predictive model.

Further work is required, especially the inclusion of more imaging data and cross-correlation of models generated, and development of new models that include more features (see section 5.3).

5.2 Impact on livestock breeding and returns

In this section we have quantified the financial benefits associated with increasing pregnancy rates and thus live calf rates in MOET programs, but this can be readily adopted for IVF and JIVET programs.

Higher and more reliable pregnancy rates are associated with:

a) Lower direct costs per MOET calf born

b) Increase in proportion of high value progeny (MOET vs. conventional or AI mating offspring)

c) Reduced recipient costs as fewer recipients are required to achieve the same number of MOET progeny

d) Shorter whole of herd calving spread

e) Increase rates of genetic gain with benefits accruing through the supply chain over time.

Direct embryo production costs have been estimated at $186 per transferable embryo. Direct embryo transfer costs have been estimated at $77. These estimates do not account for any recipient management costs or travel for practitioners. As the proportion of pregnant recipients increases, the direct cost for each calf declines (Table 1). Over a range of pregnancy rates modelled with a 5% improvement in pregnancy establishment, a reduced direct cost per calf born of between $21.92 (75% to 80% MOET pregnancy rate) to $73.06 (40% to 45% MOET pregnancy rate)(Table 1) is found.

Table 1. Direct costs per MOET calf born for pregnancy rates ranging from 40% to 80%

MOET pregnancy rate (%)	$ cost per MOET calf born
40	657.50
45	584.44
50	526.00
55	478.18
60	438.33
65	404.62
70	375.71
75	350.67
80	328.75

The largest observable benefit from increasing MOET pregnancy rate is an increase in proportion of high value stock. Industry values for contract MOET calf production suggest a MOET calf at weaning is valued at $600 higher than a calf resulting from a conventional or AI mating. That is, a contract breeder is likely willing to pay $1800 per live calf at weaning whereas other weaners are likely only valued at $1200. Therefore, in a 100 cow recipient herd, for every 5% increase in MOET pregnancy rate (calf weaning rate), e.g. from 50% to 55% there is a $3000 increase in income for contract breeding. We believe the objective assessment of embryos approach that is the focus of this project will conservatively enable a 5% increase in pregnancy rate. This will be achieved without any increase in direct embryo production or transfer costs beyond those associated with equipment purchase and embryo image capture and analysis.

As such, we propose the value proposition for MOET service providers and their clients to invest in objective image assessment of embryos is built on the assumption of a $600 premium per calf born (as informed by current contract breeding rates).

The following assumptions have been made to inform the value proposition to industry. Please note these are for illustrative purposes only and is based on an ideal workflow.

1. Equipment required to record images $15000
2. Software licence $5000 irrespective of service provision scale
3. Human resources per embryo image collected $5 (with optimised workflow)
4. MOET facility scale of 1000, 5000, 10000 embryos transferred per year

Note: the equipment does not need to be replaced on an annual basis.

As service provider scale increases the value proposition for investment is more compelling. For smaller scale service providers there is minimal benefit beyond the direct costs associated with

equipment purchase and image collection and analysis. However, for medium to larger scale service providers the benefit is significant. For a business transferring 10000 embryos per year an estimated $55,000 expenditure + $15,000 investment in capital equipment is predicted to be associated with a $300,000 increase in combined calf crop value generated. Importantly, as further images become available predictions can be further improved and even greater increases in pregnancy rate from current levels can be expected.

Table 2: Value proposition for the MOET embryo with 5% pregnancy rate gain by imaging.

Number of embryos transferred annually	Equipment cost ($)	License fee ($)	Image collection cost ($)	Increase in MOET progeny born @ 5% improvement in pregnancy rate	Increased value of MOET progeny at weaning
1000	15000	5000	5000	50	30000
5000	15000	5000	25000	250	150000
10000	15000	5000	50000	500	300000

We believe this value proposition highlights significant potential returns to industry. Importantly, it can reasonably be expected to increase uptake of MOET due to higher reliability and will lead to greater rate of genetic gain. Overall, there is considerable potential return to industry from this project. This is through returns to directly to service providers and their bull breeding clients and to the wider production sector and value chain through greater rates of genetic gain.

5.3 Requirement for further work and prediction improvement

In every respect, the grant outcomes were achieved, and the results described are hugely encouraging. The aim was to explore if imaging of 500 embryos to assess if predictive analysis was feasible. This was; but the limits that remain are that the models were built from a data set gathered over a short period of time (Nov 2017 – March 2018). There is a need to provide a generalising capability, in other words, to extend how the model will perform with new data, not included in the existing models and from a range of sources. Further data will aid us with regard to the potential improvements we might expect. These analyses are typically referred to as cross validation exercises with performance forecasting.

Improving the predictive capacity is best served by the addition of more data. One reason for this potential performance increase is that the amount of data imposes limitations in the number of informative features we should include in a robust model, this is determined by the statistical nature of the model building process itself. As we increase the amount of data, we can use more features in the model, hence we can introduce more predictive information into the model. Typically, a rule of thumb equation is employed where the number of features selected should not exceed the square root of the amount of data within the most poorly represented group, which here is the non-pregnant class. In the data set we have 163 embryos in the empty group, hence our above scientific analysis employing all data conservatively used 12 features.

Using JIVET embryos alone in the analysis we were able to see ~8-9% increase in predictive power (96% vs 88%). This fact combined with the possibility of using many more features (when more data

is available) is an excellent indication that we can achieve a real world embryo assessment system that works at the ~90% predictive level.

5.4 Achievement against grant objectives

1. Test of the accuracy with which the algorithms developed from GLCM analysis and of custom-features analysis of day 7 embryo images can predict pregnancy rates from transferred embryos

   GLCM algorithms had limited predictive capability (71%), whereas for all embryos, using custom-algorithms, a predictive capacity of 88.5% was achieved.

2. Assessment of the feasibility and practicality of capturing appropriate images for analysis in the field in a commercial setting.

   This was achieved. The microscopy system was installed and training achieved in 2 d. At times the work flow meant not all embryos could be imaged, but nevertheless, this improved with customizing the laboratory equipment (e.g. novel 'slide holder'). There is scope to custom build equipment that would streamline even further.

3. Quantification of potential cost savings (per live calf born) and logistics benefits of objective assessment of embryos prior to transfer.

   We quantified that for every 1000 embryos transferred, if a 5% improvement in pregnancy rate was achieved, a $30,000 improvement in returns to the breeder.

4. Test of the accuracy with which the algorithms developed from GLCM analysis and of custom-features analysis of day 7 embryo images can predict pregnancy rates from transferred embryos

   GLCM algorithms had limited predictive capability (71%), whereas for all embryos, using custom-algorithms, a predictive capacity of 88.5% was achieved. In particular, high predictive scores for JIVET embryos was observed (96%).

6 Conclusions/recommendations

- A scientific analysis and model building exercise using all the data available suggests we can expect to achieve predictive score of ~88% for MOET embryos and 96% for JIVET embryos, using the best features available from Quantitative's current custom feature set.
- The model was limited to a combination of eight optimally chosen features due to the limited amount of data that was available for the classification testing. However, the result provides evidence that we are on track to building a highly robust predictive model with more data available.
- We have demonstrated an unsupervised clustering model that is able to detect JIVET embryos vs MOET embryos, the JIVET forming their own cluster within our feature space, suggesting our methods have additional resolving and predictive power.
- With more data we will be in a position to effectively employ more sophisticated algorithms, and may also combine the unsupervised and supervised knowledge.

- For future development, and based on the above results we recommend at least an additional ~1700 embryo images be provided and preferably able to balance up the two groups. An additional 700 NP (non pregnant) embryos and 540 P embryos. This would provide enough data to cross validate a model employing up to 30 features.

7 Key messages

- For elite beef breeders, adoption of embryo imaging technology will improve the pregnancy rates following embryo transfer associated with female focussed advanced reproductive technologies.

- Proof-of-concept has been achieved for this technology.

- The adoption of this technology will increase the capacity for rapid genetic improvement when coupled with genomic selection.

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