Beef Cattle Breeding Project Foundation Herd: Effect of Embryo Grade and Development Stage on Pregnancy Rates

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D. Wilson, G. Rouse, D. Maxwell, R. Sealock,
and D. Kelley
Iowa State University
D. Faber, D. Broek, and M. Wehrman
Trans Ova Genetics, Inc., Sioux Center, IA

Introduction

A new beef cattle breeding project has been initiated at Iowa State University. The project has required a complete repopulation of the breeding herd. Part of the foundation cow herd has come from the purchase of virgin heifers in the fall of 1996, the remainder of the herd will come from an embryo transfer (ET) program that was conducted in the summer of 1997. The purpose of this report is to summarize some of the results of this program as they relate to blastocyst stage, embryo grade, and other factors and to summarize the resulting pregnancy rates.

Materials and Methods

Recipients for the ET program were heifers and cows left over from the previous and recently completed beef cattle breeding project. There were 81 Rhodes Research Farm spring-born 1996 heifers taken to the McNay Research Farm and commingled with the McNay heifers (89) to prepare them for the ET program. Mature cow recipients were kept at their respective farm of origin. In total, there were 170 heifers at McNay, 250 two-year-old and older cows at Rhodes, and 216 two-year-old and older cows at McNay prepared to serve as recipients. Iowa State University contracted with Trans Ova Genetics to help plan, coordinate, and perform the embryo transfers. The embryos came from three different Angus breeder sources. The majority of the embryos implanted were frozen; the balance were fresh. All females implanted with embryos were estrus synchronized according to procedures summarized in another research progress report (in preparation). The transfers at Rhodes began June 18, 1997 and ran through July 8, 1997. The transfers at McNay began June 18, 1997 and ran through July 18, 1997. The recipients at Rhodes were checked for pregnancy using ultrasound on July 30, 1997. The recipients at McNay were hand palpated for pregnancy on August 20 and September 10, 1997. Unknown pregnancy results for six embryos were deleted from the analysis in this paper.

A pregnancy result is categorical, being either pregnant (P) or open (O). Statistical significance tests of different effects on pregnancy results were accomplished using the PROC FREQ – CHISQ option of SAS (SAS User's Guide: Statistics, Ver. 5 Ed., 1985). These tests include both chi-square (χ^2) and likelihood ratio chi-square statistics.

The embryos were graded according to the International Embryo Transfer Society's guidelines for grading embryos. They were evaluated for two criteria, stage and grade. The stage of the embryo is determined by the number of cells the inner cell mass contains. A stage code is assigned for each embryo: Stage 4 – Morula, Stage 5 - Early Blastocyst , Stage 6 – Blastocyst, Stage 7 - Expanded Blastocyst, and Stage 8 - Hatching Blastocyst. Most of the donors collected were done on day 7 or 7 1/2 following breeding. The embryos will most frequently be stage 4, 5, 6, or 7 at this time. These are the ideal stages to freeze or transfer embryos.

The second criterion for grading is quality of the embryo. Quality is based on the color, texture, cohesiveness of the cells, presence of extruded cells, presence of tears in the zona pellucida, and so forth. The more imperfections the embryo has, the lower it is graded. Grade 1 embryos will be nearly perfect with few or no imperfections; Grade 2 embryos will have slight imperfections but the inner cell mass is still good; Grade 3 embryos will have many imperfections and the cell mass is usually smaller than a grade 1 or 2. The majority of the embryos in this project were grade 1 and 2 embryos. There were several grade 3 embryos as well. There were two Trans Ova Genetics embryologists (E1 and E2) that transferred the embryos.

A series of photographs taken of embryos at different stages of development follows (Figure 1-4). These photographs are provided courtesy of Trans Ova Genetics.

Figure 1. A stage 4 embryo, grade 2.

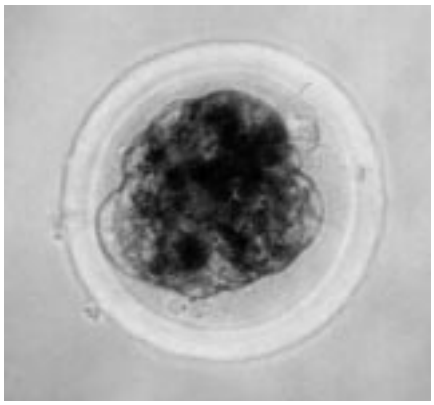


Figure 2. A stage 5 embryo, grade 1.

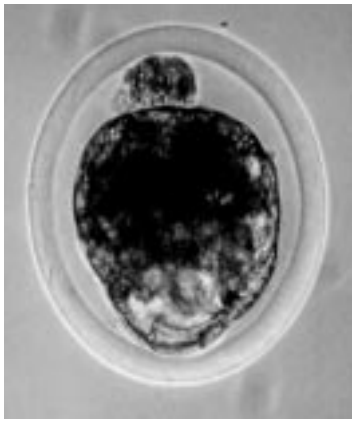


Figure 3. Two stage 7 blastocysts, grade 1 (one is collapsed).

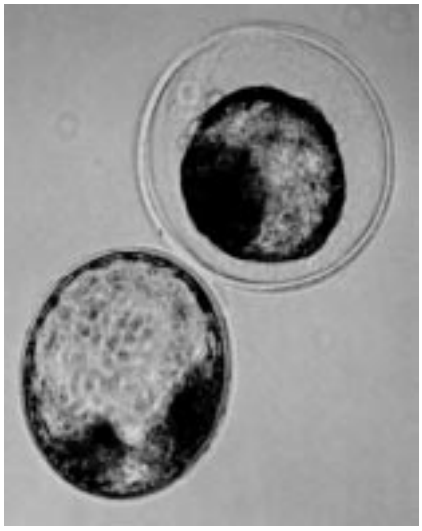
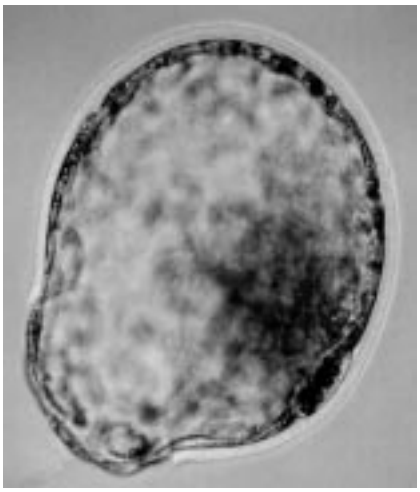


Figure 4. A stage 8, grade 1 (hatching blastocyst).



Embryos arrived at the two farm locations in three different states: fresh (F), frozen in a glycerol cryoprotectant solution (G), and frozen for direct transfer (DT). The embryos came from three ranch sources (R1, R2, and R3). All sources provided frozen embryos; two of the sources provided fresh embryos.

The embryos received from source R1 were washed and transferred into AB Technology Holding media (special preparations for embryos that will be held out of the cow for some time). They were loaded into cryoamps containing holding media and these cryoamps were secured in a small case designed to keep them upright. The case was sealed and placed in a container with gel packs that had been cooled to refrigerator temperature (4°C). This container was transported by plane from Dodge City, KS, to Chariton, IA, a flight of approximately 2 hours and 30 minutes. On arrival at the farm, the embryos were unloaded from the cryoamp and reloaded into 1/4 ml straws. These straws were placed in cassou guns and the transfers completed shortly after arrival. The total time out of the cow for the embryos was within a range of 4–8 hours.

The embryos from R2 were also transported in AB Holding media, but they were loaded individually directly into 1/4 ml straws. The embryos were placed in the center of the medium filled straws and put into containers in a horizontal position for the car ride to Rhodes, with an approximate enroute shipping time of 2 hours. They were loaded into cassou guns immediately on arrival and transferred. The total time out of the cow was within a range of 3–8 hours for different embryos.

Results

The distribution of embryos by type (frozen or fresh), stage, grade, and state is presented in Table 1. There were a total of 289 DT, 127 G, and 120 F embryos with known

pregnancy results. Within each type, the highest embryo stage frequency was 4. The distribution of these stages is purely random as it depends on when the donors are collected. Collection will occur at day 7, 7½, or on day 8. Trans Ova Genetics prefers to collect on day 7 when most embryos will be in the stage 4 or morula stage. Embryos in this stage tend to yield the highest pregnancy rates and freeze the best. Generally, there will not be many embryos that are classified as stage 5, as this is a transitional stage from morula to blastocyst. Because the embryos in this study would have been graded by more than one technician, it is difficult to know whether or not embryo stage is truly a significant factor in pregnancy results.

There were a large number of both grade 1s and 2s in each of the types of embryos. For the G type of embryo, there were 61.42% grade 2s and only 32.28% grade 1s. The highest percentage of the embryos were of stage 4 development, being 64.01, 58.33, and 81.1% for DT, F, and G, respectively.

Overall pregnancy results are shown in Table 2. The results are summarized by the source and type (fresh or frozen) of embryos. Generally speaking, the results are very similar with the frozen embryos. A 78.31% pregnancy rate was achieved with fresh embryos from source R1; however, the pregnancy rate from source 2 was 48.65%.

Overall pregnancy results by embryo grade, stage, and type (frozen or fresh) are summarized in Table 3. Pregnancy rates that are significant are printed in bold. Stage 5 resulted in the highest pregnancy rates for DT and F embryos. There were too few stage 5 embryos in the type G to see a similar result as most of these embryos were of stage 4.

The distribution of fresh embryos by stage from the two ranch sources R1 and R2 is given in Table 4. The majority of the fresh embryos from R1 were in stage 4 development with declining percentages in stages 5, 6, and 7, respectively. The majority of the embryos from R2 were also of stage 4; however, there were only 2 stage 5 embryos, with 40.54% of the embryos being stage 6.

Statistical tests of significance for morula and blastocyst stages and pregnancy rates are presented in Table 5. The results indicate that pregnancy results were significantly different ($P<.034$) for different blastocyst stages at time of transfer. The highest pregnancy rates were achieved with stage 5 blastocysts followed by stage 4, 6, and 7, respectively.

Statistical tests of significance for embryo grade and pregnancy rates are presented in Table 6. The results would indicate that grade was not a significant factor ($P<.127$) for pregnancy rate. The tendency would be, however, that grade 3 embryos, having only a 43.75% pregnancy rate, were not as suitable as either grade 1 or 2 embryos.

Statistical tests of significance for type of embryo (fresh or frozen) and pregnancy rates are presented in Table 7. The fresh embryos were analyzed by ranch source, because the preparation for transport differed between the two. Pregnancy rates were significantly different ($P<.006$) for the types of embyros. The highest pregnancy rates (78.31%) were achieved with fresh embryos received from source R1 followed by glycerol embryos, direct transfer embryos, and fresh embryos from source R2.

Statistical tests for significance for embryologist (E1 and E2) and pregnancy rates by morula and blastocyst stage are presented in Table 8. Pregnancy rate differences between the two embryologists were not statistically different by embryo stage ($P<.126$) or overall ($P<.213$).

Implications

The costs of embryo transfer may be high compared to other means of incorporating genetics in a beef cattle breeding program. But advances in technique have made this technology very competitive from a pregnancy rate standpoint compared to both natural service and artificial insemination (AI). The results from this project suggest that fresh embryos yield the best pregnancy results provided the embryos are prepared in a manner that exposes them to the least amount of stress and the least amount of time from flush to transfer. If they are to be held out of the cow for an extended period, the embryos should be cooled down for the transport period or holding period to slow down their metabolism. Additionally, care should be taken not to expose the container carrying the embryos directly to a frozen ice pack. The fluid in the container must be cooled but not frozen.

No differences in pregnancy rates were observed between Grade 1 and 2 embryos. Grade 3 embryos should not be used. If Grade 3 embryos are purchased, they should be heavily discounted on a price basis. The early blastocyst stage 5 embryos achieved the highest pregnancy rates in this project, followed closely by stage 4 or morula embryos. Based upon the pregnancy results in this study, every effort should be made to time embryo collection and transfer to achieve stage 4 and 5 embryos and to avoid stage 6 and 7 embryos. This means collecting the embryos at day 7.

Acknowledgements

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Kalamazoo, MI, in the donation of synchronization drugs used in 640 recipient females at Rhodes and McNay. The authors also extend this same grateful acknowledgement to Accelerated Genetics in the donation of beef semen for the tester herd located at McNay.

Table 1. Numbers and percentages of transferred embryos by type, stage, and grade.

Grade/Stage	4	5	6	7	Total
Direct Transfer (DT)					
1	84	31	44	0	159
	(29.07)[a]	(10.73)	(15.22)	.0	(55.02)
2	95	18	8	1	122
	(32.87)	(6.23)	(2.77)	(.32)	(42.21)
3	6	2	0	0	8
	(2.08)	(.69)	(.0)	(.0)	(2.77)
Total DT	185	51	52	1	289
	(64.01)	(17.65)	(17.99)	(.35)	(100.00)
Fresh (F)					
1	39	12	24	2	77
	(32.50)	(10.00)	(20.00)	(1.67)	(64.17)
2	31	9	0	3	43
	(25.83)	(7.50)	(.00)	(2.50)	(35.83)
Total F	70	21	24	5	120
	(58.33)	(17.50)	(20.00)	(4.17)	(100.00)
Glycerol (G)					
1	39	0	1	1	41
	(30.71)	(.00)	(.79)	(.79)	(32.28)
2	58	9	11	0	78
	(45.67)	(7.09)	(8.66)	(.00)	(61.42)
3	6	1	0	1	8
	(4.72)	(.79)	(.00)	(.79)	(6.30)
Total G	103	10	12	2	127
	(81.10)	(7.87)	(9.45)	(1.57)	(100.00)

[a]Column percentages within type of embryo are given in parentheses.

Table 2. Embryo pregnancy rates by embryo ranch source and type of embryo (frozen or fresh).

	DT		F		G	
Source	Open	Pregnant	Open	Pregnant	Open	Pregnant
R1	4	7	18	65	30	70
	(36.36)[a]	(63.64)	(21.69)	**(78.31)**	(30.00)	(70.00)
R2	42	92	19	18	8	18
	(31.34)	(68.66)	(51.35)	(48.65)	(30.77)	(69.23)
R3	60	84	0	0	0	1
	(41.67)	(58.33)				(100.00)
Total	106	183	37	83	38	89

[a]Row percentages within ranch source and type of embryo are given in parentheses.

Table 3. Overall pregnancy results by embryo grade, stage and type.

Grade/Stage	4		5		6		7	
	O	P	O	P	O	P	O	P
Direct Transfer (DT)								
1	28	56	9	22	25	19	0	0
	(33.33)	(66.67)	(29.0)	(**70.97**)	(56.82)	(43.18)		
2	34	61	4	14	2	6	0	1
	(35.79)	(64.21)	(22.22)	(**77.78**)	(25.0)	(75.0)		(100.0)
3	3	3	1	1	0	0	0	0
	(50.0)	(50.0)	(50.0)	(50.0)				
Overall	65	120	14	37	27	25	0	1
	(35.14)	(64.86)	(27.45)	(**72.55**)	(51.92)	(48.08)		(100.0)
Fresh (F)								
1	12	27	1	11	9	15	1	1
	(30.77)	(69.33)	(8.0)	(**91.67**)	(37.5)	(62.5)	(50.0)	(50.0)
2	11	20	1	8	0	0	2	1
	(35.48)	(64.52)	(11.11)	(**88.89**)			(66.67)	(33.33)
Overall	23	47	2	19	9	15	3	2
	(32.86)	(67.14)	(9.52)	(**90.48**)	(37.5)	(62.5)	(60.0)	(40.0)
Glycerol (G)								
1	9	30	0	0	1	0	0	1
	(23.08)	(**76.92**)			(100.0)			(100.0)
2	18	40	3	6	2	9	0	0
	(31.03)	(**68.97**)	(33.33)	(66.67)	(18.18)	(81.82)		
3	3	3	1	0	0	0	1	0
	(50.0)	(50.0)	(100.0)				(100.0)	
Overall	30	73	4	6	3	9	1	1
	(29.13)	(**70.87**)	(40.0)	(60.0)	(25.0)	(75.0)	(50.0)	(50.0)

Table 4. Distribution of fresh embryos by stage from the ranch sources.

Source/Stage	4	5	6	7	Total
R1	50	19	9	5	83
	(59.52)[a]	(22.62)	(10.71)	(1.19)	
R2	20	2	15	0	37
	(54.05)	(5.41)	**(40.54)**		
Overall	70	21	24	5	120
	(57.85)	(17.36)	(19.83)	(4.13)	

[a]Percentages in parentheses are row percentages.

Table 5. Pregnancy results by morula and blastocyst stage.

Stage/Pregnancy	Open	Pregnant	Total
4	118	240	358
	(32.96)[a]	(67.04)	
5	20	62	82
	(24.39)	**(75.61)**	
6	39	49	88
	(44.32)	(55.68)	
7	4	4	8
	(50.0)	(50.0)	
Statistic	Probability		
Chi-Square (χ^2)	.034		
Likelihood Ratio χ^2	.035		

[a]Percentages in parentheses are row percentages.

Table 6. Pregnancy results by embryo grade.

Grade/Pregnancy	Open	Pregnant	Total
1	95	182	277
	(34.30)[a]	(65.70)	
2	77	166	243
	(31.69)	(68.31)	
3	9	7	16
	(56.25)	**(43.75)**	
Statistic	Probability		
Chi-Square (χ^2)	.127		
Likelihood Ratio χ^2	.143		

[a]Percentages in parentheses are row percentages.

Table 7. Pregnancy rates by type of embryo.

Type/Pregnancy	Open	Pregnant	Total
Direct Transfer (DT)	106	183	289
	(36.68)[a]	(63.32)	
Fresh – Ranch 1	18	65	83
	(21.69)	**(78.31)**	
Fresh – Ranch 2	19	18	37
	(51.35)	(48.65)	
Glycerol (G)	38	89	127
	(29.92)	(70.08)	
Statistic	Probability		
Chi-Square (χ^2)	.006		
Likelihood Ratio χ^2	.006		

[a]Percentages in parentheses are row percentages.

Table 8. Pregnancy results by embryologist.

	Total Number	Pregnancy Rate, %	Probability, χ^2
Stage 4			
E1	297	68.35	
E2	61	60.66	.244
Stage 5			
E1	69	72.46	
E2	13	92.31	.126
Stage 6			
E1	65	60.00	
E2	23	43.48	.170
Overall			
E1	439	67.43	
E2	97	60.82	.213