Past, Present and Future Genetic Embryo testing in Cattle

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Historical

Sex determination of bovine embryos by restriction fragment polymorphisms of PCR amplified ZFX/ZFY loci.

Pollevick GD[1], Giambiagi S, Mancardi S, de Luca L, Burrone O, Frasch AC, Ugalde RA. Biotechnology (N Y). 1992 Jul;10(7):805-7.

Sensitive sex determination assay applicable to bovine embryos derived from IVM and IVF.

Kirkpatrick BW[1], Monson RL. J Reprod Fertil. 1993 Jul;98(2):335-40.

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Why biopsy

- Cost of gestating unwanted genetics
  - $ values of unwanted calves often females only desired
  - Recipient costs

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In vivo Flush Embryo Biopsy Sexing

- Late 1990s early 2000s
- ~75% of *in vivo* embryos were biopsied per flush
- 4-5 cells are needed for PCR
- On average 10% failure rate to generate a sexing result
- Fresh embryos were transferred 2-4 hours after biopsy and results (PCR and agarose gel)
- Slight 5-10% decrease in pregnancy rates
- Embryos implanted costs 2x to produce and 2x test

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Industry trends

- Best embryos are biopsy candidates ~75%
- What do with other 25%
- Viable embryos that can not be tested are not high enough quality to freeze
- Cost of embryo production
- Impact on pregnancy rate
- Cost benefit decision models

- Change in technology sexed semen becomes available

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Screening embryos for genetic defect carriers embryos

- Parent known carrier of genetic defect
- Biopsy and testing option to create clean progeny

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Biopsy and genomic enhanced genetic merit values - embryo selection

Assessment of MDA efficiency for genotyping using cloned embryo biopsies

Lauri et al. Genomics 101 (2013) 24–29

Impact of whole-genome amplification on the reliability of pre-transfer cattle embryo breeding value estimates

Shojaei Saadi et al. BMC Genomics 2014, 15:889

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What needed for biopsy and HD genotyping

- Whole Genome Amplification
- Freezing of biopsied *in vivo* and IVF embryos for implant after receiving results
- Turn around times for results reasonable 30-40 days
- Pregnancy rates that are not compromised to much

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R&D sorting embryos from pooled oocytes after fertilization and culture

Animal ID	call rate	Dam excluded	Disconcordance	opposite	hetero,homo	AY76113	AY77615	AY84115	AY84247	AY84247
1.1.7Fetal DNA	95	0	22	3	19	A/T	A/A	G/T	G/G	C/C
1.1.7t biopsy	77	2				A/T	A/A		G/G	
1.1.48Fetal DNA	96	0	11	0	11	A/T	A/G	T/T	G/G	C/C
1.1.48t biopsy	85	2				A/T	A/A	T/T	G/G	C/C
1.1.60Fetal DNA	95	0	10	1	9	T/T	A/G	G/T	A/G	C/G
1.1.60t biopsy	89	3				T/T	A/A	T/T	A/G	C/G

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R&D biopsy Whole Genome Amplification

- Create "fake biopsy" using cells lines (10-12 cells per biopsy)
- Replicates of 3 biopsy per cell line
- Control DNA extracted from cell line equals tissue sample from calf

Sample		call rate	Total comparable	Total correct	Total erro	%error	Missing Geno
1RF		87	57764	55781	1983	3.43%	8957
2RF		90	60622	59913	709	1.17%	6799
3RF		89	60302	59285	1017	1.69%	7436
1R-control	66721	99					

Sample	HeteroC	HomoC	het/homo Error	homo/het Error	oppositehomo
1RF	6571	49210	278	1698	7
2RF	8983	50930	119	590	0
3RF	8274	51011	97	916	4

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Results Confirm Parental Correctional Imputation Needed

- Shojaei Saadi et al. BMC Genomics 2014, 15:889

- Cost benefit analysis
  - 60-70% of IVF embryos produced can be biopsied
  - Remaining embryos are not freezable
  - Preg rate drop ~5-15% for biopsy frozen embryos
  - ~$40 genomic test, $50 freezing costs, Biopsy and WGA $.

4 years ago ~600 embryos were genomic tested in Canada (Reported by CETA)
The last 2 year less than 200 per year have been genomic tested in Canada.

Current status

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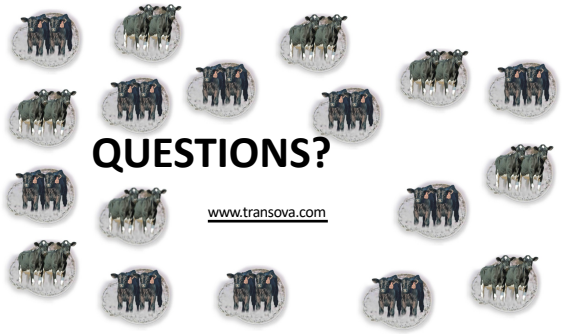
THANK YOU



QUESTIONS?

www.transova.com

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