Sex-specific Early Development in Bovine Embryos

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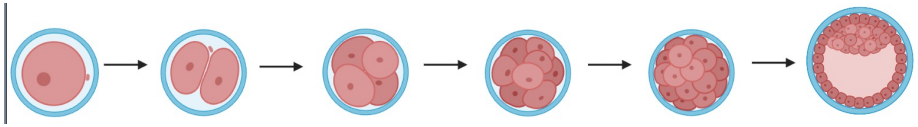
Background – what causes the sex difference in embryogenesis?

Preimplantation embryonic development

- Bovine and human embryos share various biological similarities.
- Avoids ethical issue of using human embryos.



Zygote 2-cell 4-cell 8-cell Morula Blastocyst

D: 1 2 3 4 5-6 7-8

- Blastocyst is the organism's first cell differentiation

Sexual dimorphism among bovine embryos in their ability to make the transition to expanded blastocyst

Table 1

Development and sex ratio of bovine embryos after different times in culture in TCM-199

Collection stage	Day	Embryo number	Sex Ratio, male:female
Eight-cell	3	192	46:54
Morula	6	153	50:50
Morula	8	103	37:63*
Early blastocyst	8	79	52:48
Blastocyst	8	72	67:33*
Late blastocyst	8	42	64:36
Expanded blastocyst	8	65	83:17†

*Significantly different from anticipated 1:1 ratio of males to females ($P < 0.05$).

† Significantly different from anticipated 1:1 ratio of males to females ($P < 0.01$).

Cleavage rate: Males > Females

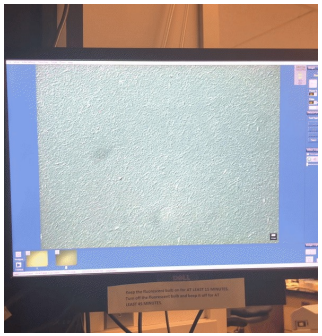
Scientists observed sexual dimorphism of developmental rates

Method

Embryology

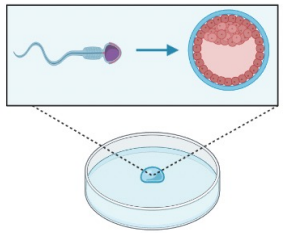
Aspirate oocyte from ovaries

Oocyte selection and maturation in IVM medium

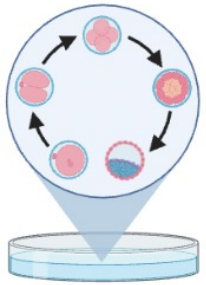


Sperm motility check

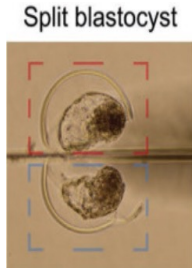
Sperm concentration check



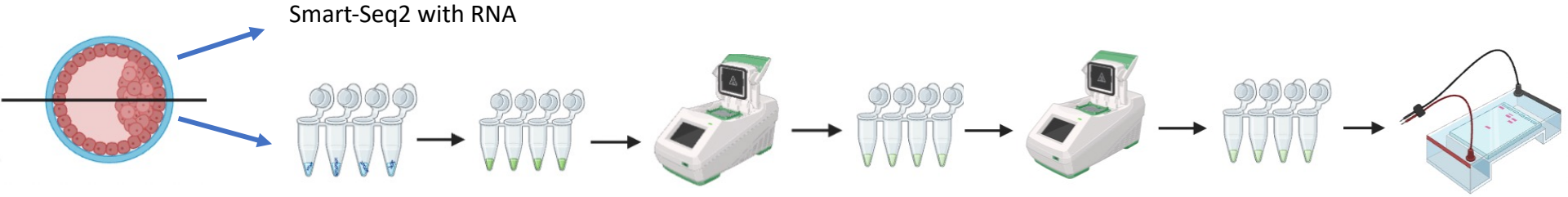
IVF in IVF media for 18 hr.



Embryo in IVC culture for 7 d



Sexing



Split blastocyst

Embryo lysis

Y-linked primer

1st PCR-20 cycle

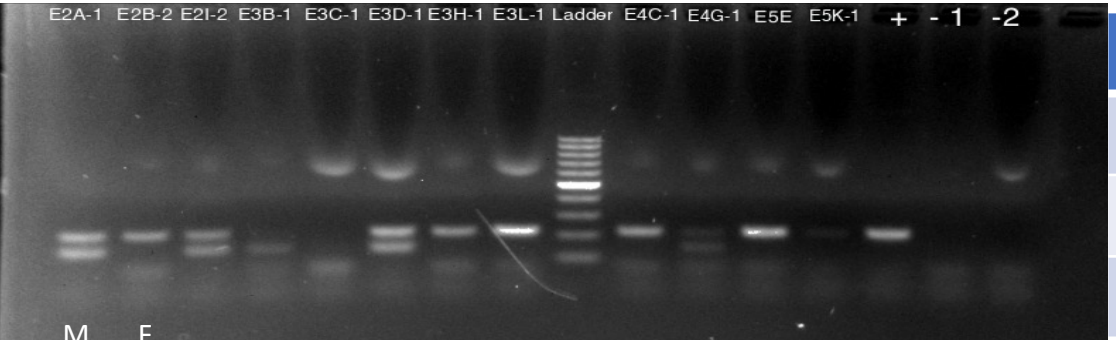
Add autosomal primer

Second PCR-17 cycles

PCR product

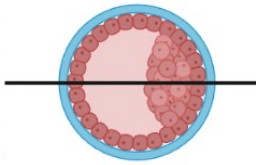
Run 2% agarose gel

Results



Sex of Day 7 and 8 Bovine Embryos			
	Male	Female	Unknown
Day 7	23	20	7
Day 8	6	2	4

Conclusion & Future Steps

- We have a slight trend skewed towards male; however, we need to collect more data to get the study analyzed.
-  ---> Smart-Seq2
- Novogene – sequencing company. CDNA libraries sent out to detect differential gene expression, alternative splicing, RNA editing and more