The morphokinetics of XY- and XX-sorted embryos

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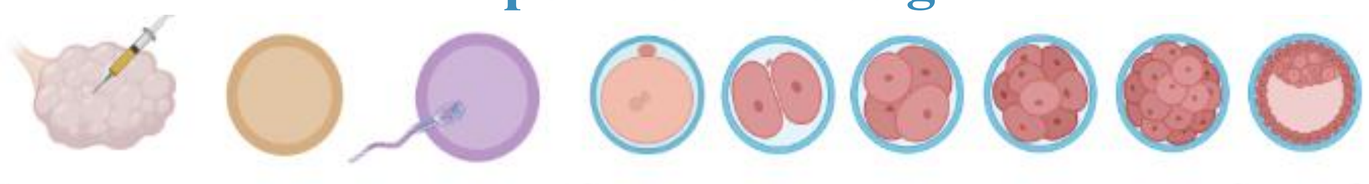
Background

- In the milk industry, the female is the more profitable gender.
- Flow cytometry is used to sort bovine semen, but it is expensive, harmful and reduces pregnancy rate.
- In humans, preimplantation genetic diagnosis (PGD) is used to determine embryo gender.
- A noninvasive method will pave the way to commercial embryo transfer with gender specification.

Objectives

1. Characterize the **morphokinetics** of male and female bovine embryos
2. Examine whether **morphokinetic** parameters can be used to predict the gender of bovine embryos

Experimental design



*COCs: cumulus cell complexes

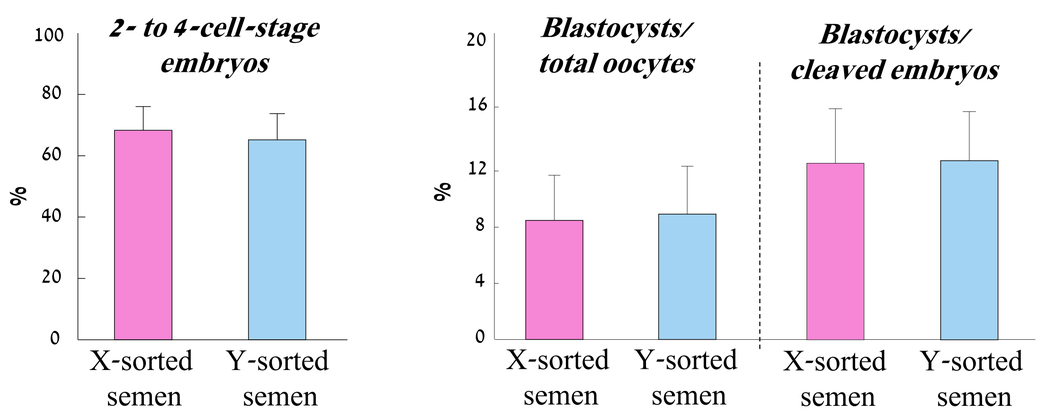
Parameters evaluated:

1. Developmental competence
2. Morphokinetics
3. Gene expression

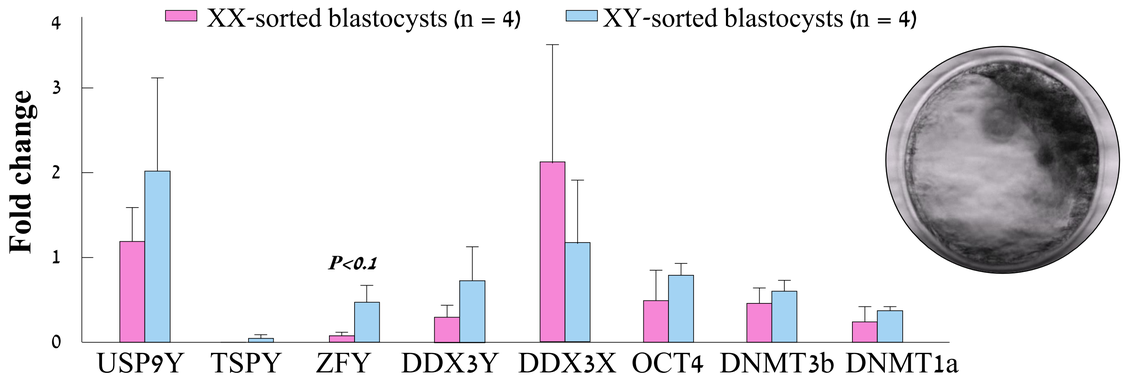
n = 1197 oocytes; 12 replicates

Results

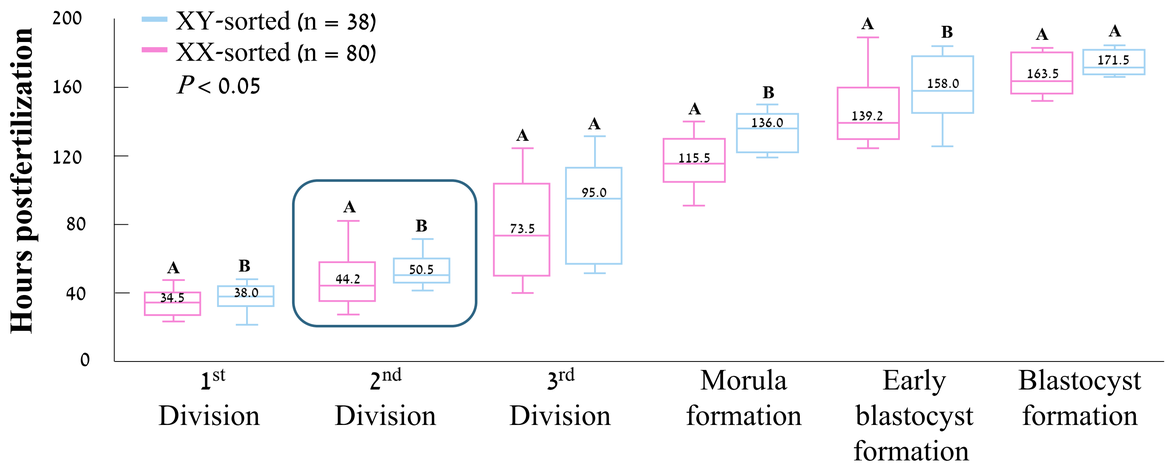
Embryonic development



Evaluation of transcript abundance in blastocysts

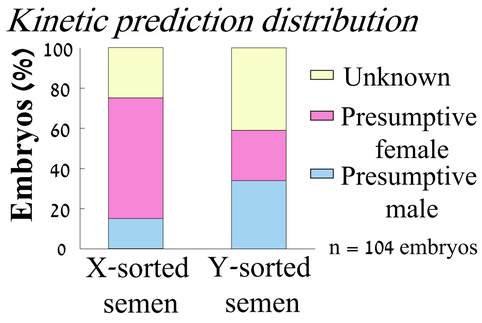


Kinetics of embryos developed from X- *vs.* Y-sorted semen

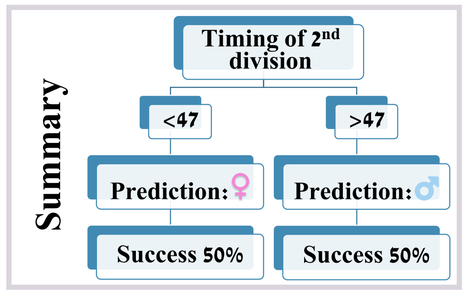


Gender prediction based on kinetics of the 2nd division

Kinetic prediction distribution



n = 104 embryos

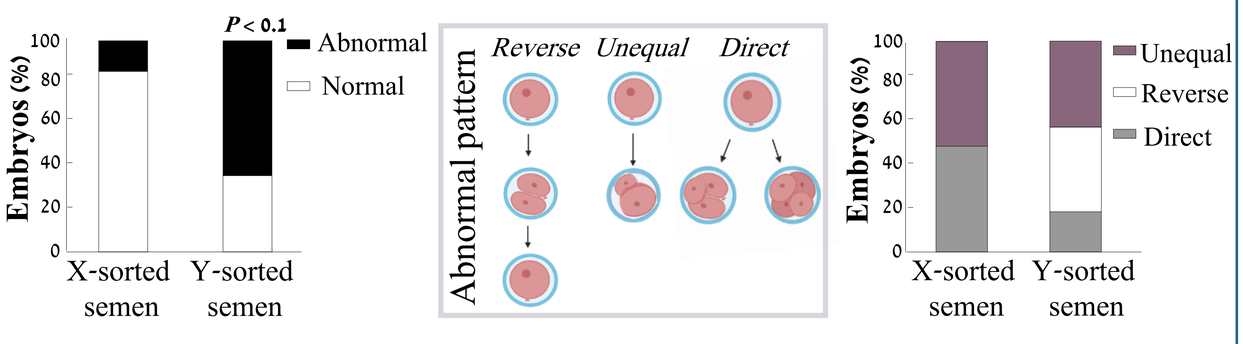


Kinetic prediction and genetic evaluation

Embryo no.	Sorted semen	Kinetics of 2nd division	USP9Y gene	DDX3X gene	Prediction
1	Y	XY+	XY+	XY+	Male
2	Y	XX+	XX+	XY+	Female
3	Y	XX+	XY+	XY+	False positive
4	Y	XY+	XY+	XX+	False positive
5	X	+/-	XX+	XY+	Unknown
6	X	XX+	XX+	XX+	Female
7	X	+/-	XY+	XY+	Unknown
8	X	XX+	XY+	XX+	False negative

+/- can be either XX- or XY-developed embryos

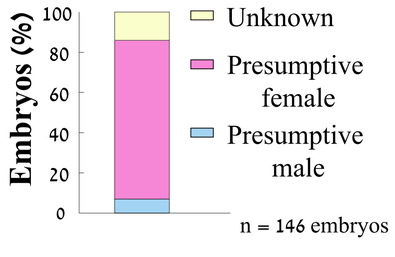
Morphokinetics of blastocysts developed from X- *vs.* Y-sorted semen



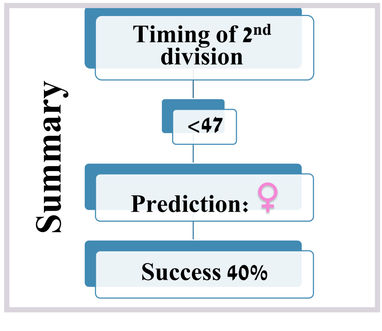
XX-sorted embryos (n = 14), XY-sorted embryos (n = 14)

Gender prediction for nonsorted embryos, based on kinetics of the 2nd division

Kinetic prediction distribution



n = 146 embryos



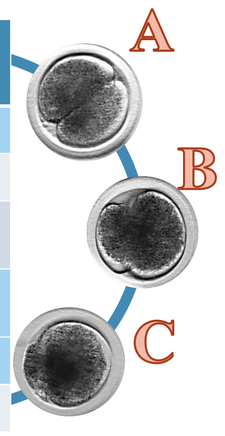
Kinetic prediction and genetic evaluation

Embryo no.	Kinetics of 2nd division	ZFY expression (yes/no)	Predicted gender
1	XX+	Yes	False positive
2	XX+	No	Female
3	XX+	Yes	False positive
4	XX+	No	Female
5	XX+	Yes	False positive
6	XX+	Yes	False positive
7	XX+	Yes	False positive
8	XX+	Yes	False positive
9	XX+	No	Female
10	XX+	No	Female

Morphology of embryos developed from X- *vs.* Y-sorted semen

Stage of development	A-grade embryos (%)		B-grade embryos (%)		C-grade embryos (%)	
	XX	XY	XX	XY	XX	XY
2 cells	86.17	80.39	13.83	19.61	-	
4 cells	75.86*	41.67*	24.14*	58.33*	-	
6 cells	78.05*	45.45*	21.95*	45.45*	0*	9.09*
8 cells	71.88	75	28.13	16.67	0	8.33
Blast	66.67	40	33.33	60	-	

*$P < 0.05$; n = 145 embryos



Conclusions

- Embryos developed from X- and Y-sorted semen differed mainly in their kinetics, especially of the 2nd division, with faster division in the XX group.
- Gender prediction based on the kinetics of the 2nd division demonstrated 40-50% success.