Sex selection

Christopher LR Barratt

Reproductive and Developmental Biology,
Medical School,
Dundee,
Scotland

Members of Lab : Marios Stavridis, Senga Oxenham, Anne Duncan, Wardah Alasmari, Vanessa Kay, Erica Forster, Stephen Mansell, Anne Rice and Cathy Beaton.

Background

- Intensive interest : since time began : www.google.com
- Numerous techniques - many 'suspect'.
- Approaches: sperm selection [pre fertilisation] and PGD [post fertilisation].
- Safety, efficacy, social questions:
  - If should be used for medical reasons?
  - If should be used for social reasons?

Only concentrate on sperm sorting and methods (not ethics)

But should we be concerned?

Absolutely

Abnormal sex ratios in human populations: Causes and consequences

Table 1. Numbers of missing females for selected Asian countries, 2001

Country	Calculated no. of missing females, in millions*
Afghanistan	0.5–1
Bangladesh	1.8–3.7
China	39–41
India	27–28
South Korea	0.7–0.1
Pakistan	2.6–4.9
Taiwan	0.4–0.6
Iran	0.8–1.2

How do we alter sex ratio?
How do we select X and Y sperm?

Old wives tales…..

Simpson & Carson

- Male/female : 1.05 (more males)
- Several methods discussed pros/cons
  - Demographics (war [more males], age, coital frequency etc)
  - Timing of intercourse and conception relative to ovulation.
  - Shettles : female child if : acidic douche, face to face intercourse, 2/3 days before ovulation …….
  - Overall conclusion ‘no clinically meaningful relationship between sex ratio and conception timing’

Table I. Sex ratio by timing of insemination relative to ovulation and planning status of the pregnancy

Timing of insemination[a] (days)	Unplanned pregnancies		Planned pregnancies		Total pregnancies	
	Sex ratio M/F	(M/F * 100)	Sex ratio M/F	(M/F * 100)	Sex ratio M/F	(M/F * 100)
≤5	89/87	102.3	12/8	150.0	101/95	106.3
−2 to −4	52/67	77.6	41/45	91.1	93/112	83.0
−1	29/16	182.5	99/38	116.8	92/4	124.5
0	23/16	143.8	87/104	83.7	110/120	91.7
+1	17/12	141.7	15/18	83.3	32/30	106.7
≥2	40/29	137.9	6/7	85.7	46/36	127.8
Total	247/227	108.8	227/240	94.6	474/467	101.5

[a]The number of days from the most probable insemination intercourse to probable day of ovulation (day 0).
M/F = Number of males per 100 females. There were 6 pregnancies of indeterminant planning status.

Sperm sorting: gradients

Pros	*Cons*
✓ No apparent increased health risk	× Not completely reliable
✓ Cheaper than more technologically intensive alternatives	× Reasons for apparent effectiveness unclear
✓ Less likely that embryos will be destroyed	× Currently unregulated, therefore no quality control

'Although more than 100 patents exist that claim successful sexing of sperm, most procedures are no more efficacious than folk methods were more than two millenia ago' Garner & Seidel 2008 Theriogenology 69,886-895.

Sperm sorting

- Gradient methods
  - Sperm placed on Colum and centrifuged [protein 'mythical X', Percoll/Ficoll]
  - Putatively separates X from Y sperm
  - Success rates remains debatable – independent verification is required and not yet [unfortunately] forthcoming.

Gradient methods don't work!

FERTILITY AND STERILITY®
Copyright © 1997 American Society for Reproductive Medicine
Published by Elsevier Science Inc.

Vol. 67, No. 6, June 1997
Printed on acid-free paper in U. S. A.

Failure of multitube sperm swim-up for sex preselection*

Christopher J. De Jonge, Ph.D.†
Sean P. Flaherty, Ph.D.‡
Annette M. Barnes, B.S.
Nicholas J. Swann, B.A.‡
Colin D. Matthews, M.D.‡

15 Minute Swim-up

B	Neat Semen (A)					Upper Fraction (B)					Lower Fraction (C)				
	% 0	% X	% Y	% XY	X:Y	% 0	% X	% Y	% XY	X:Y	% 0	% X	% Y	% XY	X:Y
Mean	1.1	48.9	49.6	0.4	0.99	0.9	49.4	49.4	0.3	1.00	0.9	49.2	49.8	0.2	0.99
S.D.	0.2	1.1	1.0	0.1	0.04	0.3	1.3	1.2	0.2	0.05	0.3	1.1	0.9	0.1	0.04
Median	1.10	49.00	49.50	0.40	0.99	0.90	49.30	49.70	0.30	0.99	0.80	48.80	49.80	0.20	0.98
Maximum	1.40	50.70	51.10	0.50	1.06	1.30	51.70	51.00	0.70	1.09	1.70	50.80	51.20	0.40	1.05
Minimum	0.80	47.30	48.00	0.30	0.93	0.40	47.80	47.30	0.00	0.94	0.60	47.50	48.50	0.00	0.94

Ericsson method (1973) doesn't work..

Flaherty *et al* (1997) Hum Reprod 12, 938-942

Table I. Separation of sperm on albumin gradients using the protocol 3 method

	Pre-separation			Post-separation				FISH analysis				
Sample	TSC	% Motility	TMS	TSC	% Motility	TMS	% TMS	% 0	% X	% Y	% XY	X:Y
1	266	63	167.6	6.5	100	6.5	3.9	1.2	49.0	48.7	0.1	1.02
2	94	50	47.0	1.2	100	1.2	2.6	1.2	47.6	50.7	0.5	0.94
3	381	87	331.5	16.4	99	16.2	4.9	1.3	47.9	50.7	0.1	0.94
4	202	48	97.0	6.2	74	4.6	4.7	1.2	48.2	50.6	0.0	0.95
5	480	73	350.4	22.0	84	18.5	5.3	1.1	51.4	47.4	0.1	1.08
6	337	67	225.8	14.0	95	13.3	5.9	0.8	49.1	50.1	0.0	0.98
7	747	77	575.2	8.7	94	8.2	1.4	0.4	53.2	45.3	1.1	1.17
8	994	52	516.9	21.0	79	16.6	3.2	0.7	52.1	46.4	0.8	1.12
9	344	67	230.5	9.2	95	8.7	3.8	0.6	51.1	48.0	0.3	1.06
10	480	62	297.6	10.7	100	10.7	3.6	1.1	51.0	47.1	0.8	1.08
11	133	76	101.1	5.8	100	5.8	5.7	0.7	43.7	55.2	0.4	0.79
Mean	405	66	267.3	11.1	92.7	10.0	4.1	0.9	49.6	49.1	0.4	1.01
SD	267	12	169.5	6.6	9.4	5.5	1.4	0.3	2.7	2.7	0.4	0.11

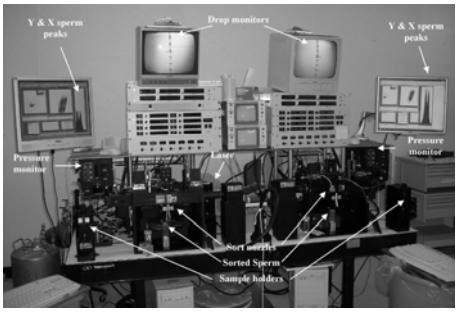
TSC = total sperm count ($\times 10^6$); TMS = total motile sperm count ($\times 10^6$).
%TMS = TMS (post-separation)/TMS (pre-separation)×100.

However (and it is a big one) for births the Ericsson method does work (maybe)

Births (n)	Treatment sex ratio (%)	Control sex ratio without cc (%)	Control sex ratio with cc (%)
Single (184)	27.7	51.4 P<0.001	48.1 P<0001
Twin (42)	33.3	50.2 P<0.01	48.1 P<0.03
Combined (226)	28.8	51.4 P<0.001	48.1 P0.001

Silverman *et al.*, 2002, Hum Reprod 17, 1254-1256

The answer -flow cytometry



–Garner & Seidel 2008 Theriogenology 69,886-895.

Sperm sorting: flow cytometry

Pros	*Cons*
✓ Relatively high success rate ✓ Sperm can be used for insemination rather than more invasive IVF ✓ Less likely that embryos will be destroyed	× Not completely reliable × Very expensive, specialised and not widely available × Question over safety for use in humans, although no contraindications from use in domestic cattle

Flow cytometry

- First live offspring –rabbits in 1989.
- Primarily used in cattle
- x/y human sperm differ in DNA (but only 2.8% small compared to some animals) and wide variation in amount of Y heterochromatin.
- Fluorescent dye added to sperm sample
- Dye binds to DNA (non intercalating)
- UV laser
- FACS sorted (hours 4 per sort on average and obtain only 30,000 cells)
- Separated sperm used for insemination or IVF
- www.Microsort.com

First babies born from MicroSort® technology .

Fugger *et al.,* (1998) Hum Reprod 13, 2367-2370.

X Sort

- Sex linked disorders or family balancing (90%).
- Used IVF and ICSI and IUI.
- IUI : average 130,000 sperm inseminated peri-ovulatory [sperm survival is affected].
- Total 29 pregnancies achieved. 9 patients given birth to 11 normal babies. 92% had only female conceptions (desired outcome).

MicroSort® most recent data

[Karabinus DS Theriogenology 2009, 71, 74-79]

- Exclusive licence to patented flow cytometric sperm separation technology.
- For IUI timed insemination with daily ultrasound and usually empiric oral clomiphene citrate.
- Inseminated 28-52 hrs after detection of LH surge or greater than 36h after hCG administration.
- Either fresh or frozen semen following DGC stained with Hoeschst 33342 final concentration 9µM
- 91.8% for Family balancing.
- Of 5871 sorts 74.9% for X, 25.1% for Y
- 1125 clinical pregnancies, 943 babies with 167 ongoing
- 1642 sorts with IVF
- 3629 sorts for IUI

Post sort purity, embryo and neonatal sex after MicroSort.
[Karabinus DS Theriogenology 2009, 71, 74-79.

X Sort	Y Sort
Sorted sperm 87.9% X n=4399	Sorted sperm 73.4% Y n=1472
Embryo sex 87.7% F n=1320	Embryo sex 65.8% M n=1314
Baby Sex 92% F n=726	Baby Sex 83.6% M n=284

PR and SAb by female age for IUI cycles with MicroSort®
[Karabinus DS Theriogenology 2009, 71, 74-79]

Age (year)	Cycles	CP n	PR/cycle %	SAb n	Clinical loss %
<30	317	64	20	6	9.4
30-34	1384	232	17	29	12.5
35-39	1780	259	15	50	19.3
>40	148	12	8	4	33.3
All	3629	567	15.6	89	15.7

Average 195,000 motile sperm inseminated. Very low.

Safety of FACS for human X/Y sorting.

- Does laser and DNA dye effect sperm : jury out??????
- Its been used for the last 20 years.
- Long term follow up in cattle not really available but over one million animals [pigs, cattle, sheep, dolphins, humans, rhinos] have been born as a result of sperm sorting (no increase in abnormalities observed to date). But reduction in fertility has been consistently observed.
- Large field trials in bovines showed no increase in abortion rate, gestation length, neonatal death, calving difficulty, birth weight or live births.
- Dye is excitation at 350nm [minor effect on DNA as not absorbed by |DNA and proteins] excitation at 460nm. Some cells are very sensitive other not [presumably high quality sperm].
- Congenital malformation rate of babies - similar to normal – 760 babies 2.6% major congenital malformations, 6.1% minor and 3.0% exhibiting variants. 'It can be stated that with 95% confidence that the MMR does not exceed 3.5%'. [Karabinus DS Theriogenology 2009, 71, 74-79]
- What evidence should we require before universally accepted?

Garner DL 2009 Theriogenology 71, 11-21 excellent discussion of safety of the dye e.g. gene expression profiles in bovine embryos changed if generated using sorted cells]

Summary

- Sperm sorting prior to IUI does enrich the proportion of X/Y sperm (FACS) and birth ratios. However its expensive, not 100%, exclusive and possible concern over safety remain although these are rapidly diminishing.
- Sperm sorting by other methods – independent verification is required.
- New methods will undoubtedly be available in the near future.