Gene Banking and Cryopreservation Training Workshop

1-3- June 2010 – CGR, Wageningen UR, Lelystad

Making the decision: from objectives to operation of gene banks

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Sources of information used for this presentation:

- **FAO Guidelines for the Cryoconservation of Animal Genetic Resources** (Ed. P. Boettcher, Draft 2010)

- **ERFP Guidelines for the Constitution of national Cryopreservation Programmes for Farm Animals** (Ed. S.J. Hiemstra, 2003)

- **Utilisation and conservation of farm animal genetic resources** (Ed. K. Oldenbroek, Wageningen Academic Publishers, 2007)

- **Scientific literature**

N.B. : In developing national guidelines / programmes, I would recommend to refer, as much as possible, to International Guidelines in order to promote some homogenisation across countries.

Gene banks primary function:

The conservation of genetic resources for their use, from short to long term

Gene banks can have a multi-function role:

- To reconstruct breeds / breeding lines;

- To support populations conserved *in vivo*:
  - to increase effective population size of small populations;
  - as a back-up in case genetic problems occur (e.g. loss of allelic diversity, inbreeding, occurrence of deleterious genetic combinations);

- To develop new lines / breeds;

- As a back-up, to quickly modify and/or reorient, the evolution /selection of populations;

- For research (DNA source; multi-generational samples of genetic variation, etc.).

Because the multifunctional role, in gene banks material could be subdivided into categories such as (FAO Guidelines):

- **core collection** – to be used in critical situations; dimension = 150% of the material needed for breed reconstruction (FAO); to be periodically updated;

- **historic collection** – material "dated" from the core collection;

- **working collection** – for research, development of new breeds/lines

- **evaluation collection** – to evaluate the status of the material

To establish a gene bank is an expensive operation, therefore planning is advisable

Operational Steps

Choice of populations

Type of material: evaluate pro and contra

Amount of material: n. of doses
n. of donors

Selection of donors

Frequency of collection

Operational Steps – Choice of populations

Factors to be considered:

- Existing collections
- Level of endangerment
- Conservation value
- Costs
- Organisational aspects
- Political aspects

Operational Steps – Type of material

Options:

- **Semen**
- **Embryos**
- **Oocytes**
- **Embryos + semen**
- **Somatic cells**

Operational Steps – Type of material
Semen only

Aims	Disadvantages	Yes/poor/no
Breed reconstruction	Backcross is a long & expensive process Genes recovered < 100% Cytoplasmatic effects lost/altered	yes
Support population conserved in vivo		yes
Develop new lines / breeds		yes
Back up to re-orient breed evolution		yes
Research	No mitochondrial DNA	yes

Operational Steps – Type of material
Embryos only

Aims	Disadvantages	Yes/poor/no
Breed reconstruction	More expensive than semen	yes
Support population conserved in vivo	More expensive than semen	yes
Develop new lines / breeds	More expensive than semen	yes
Back up to re-orient breed evolution	More expensive than semen	yes
Research	More expensive than semen	yes

Operational Steps – Type of material
Oocytes

Aims	Disadvantages	Yes/poor/no
Breed reconstruction	Need also semen Compared to embryos, possible desired mating; Costs compared to embryos ?	yes
Support population conserved in vivo	More expensive than semen	yes
Develop new lines / breeds	More expensive than semen	yes
Back up to re-orient breed evolution	More expensive than semen	yes
Research	More expensive than semen	yes

Operational Steps – Type of material
Embryos plus semen

Aims	Disadvantages	Yes/poor/no
Breed reconstruction	Cheaper than embryos and possibly of semen	yes
Support population conserved in vivo	More expensive than semen	yes
Develop new lines / breeds	More expensive than semen	yes
Back up to re-orient breed evolution	More expensive than semen	yes
Research	More expensive than semen	yes

Operational Steps – Type of material
Somatic cells

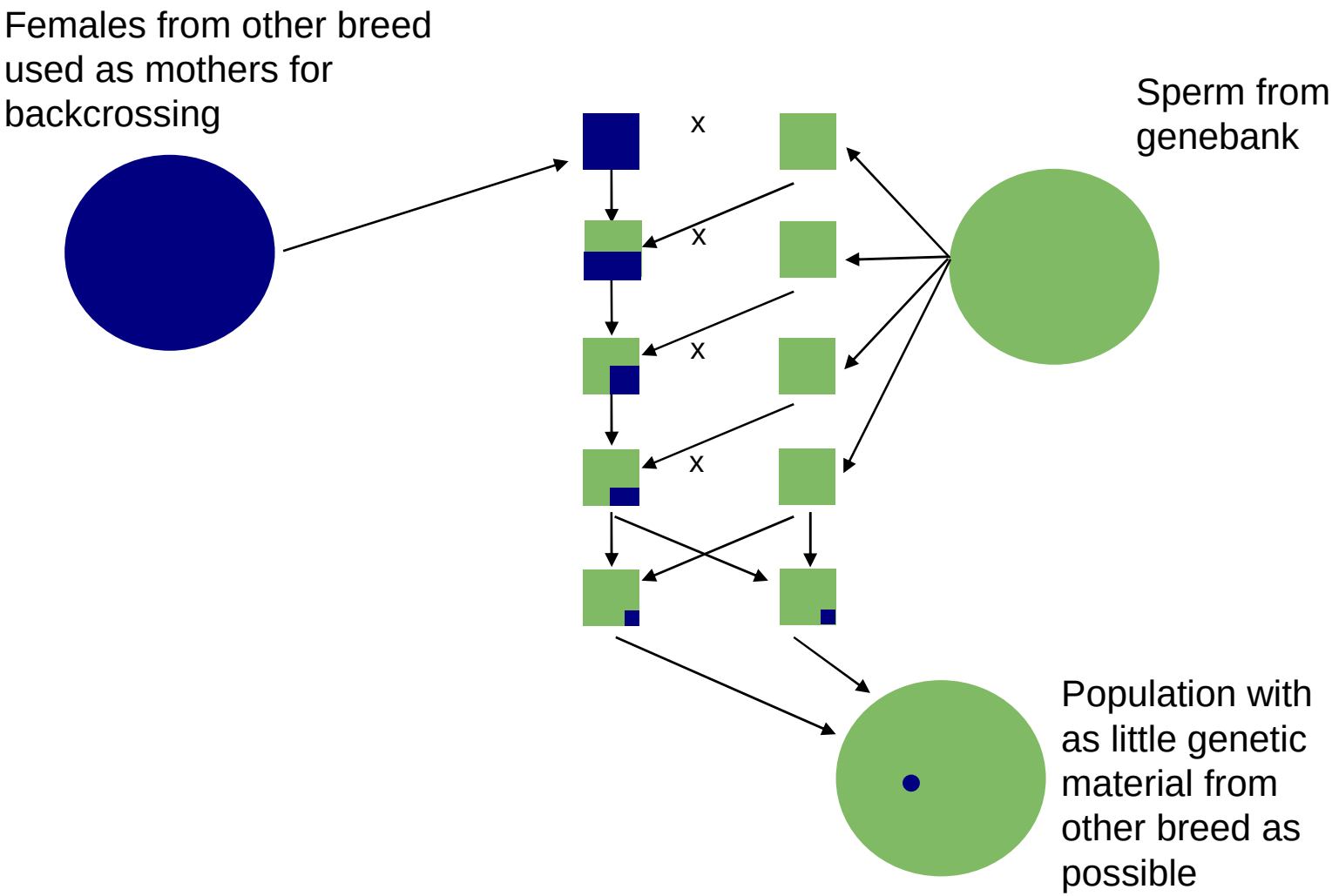
Aims	Disadvantages	Yes/poor/no
Breed reconstruction	Costs ? Genetic problems ?	yes
Support population conserved in vivo		yes
Develop new lines / breeds		yes
Back up to re-orient breed evolution		yes
Research		yes

Amount of material

Because breed reconstruction requires the largest amount, we could keep this options as reference point. This amount constitute the core collection (x 1.5).

With limited funds we must consider smaller targets

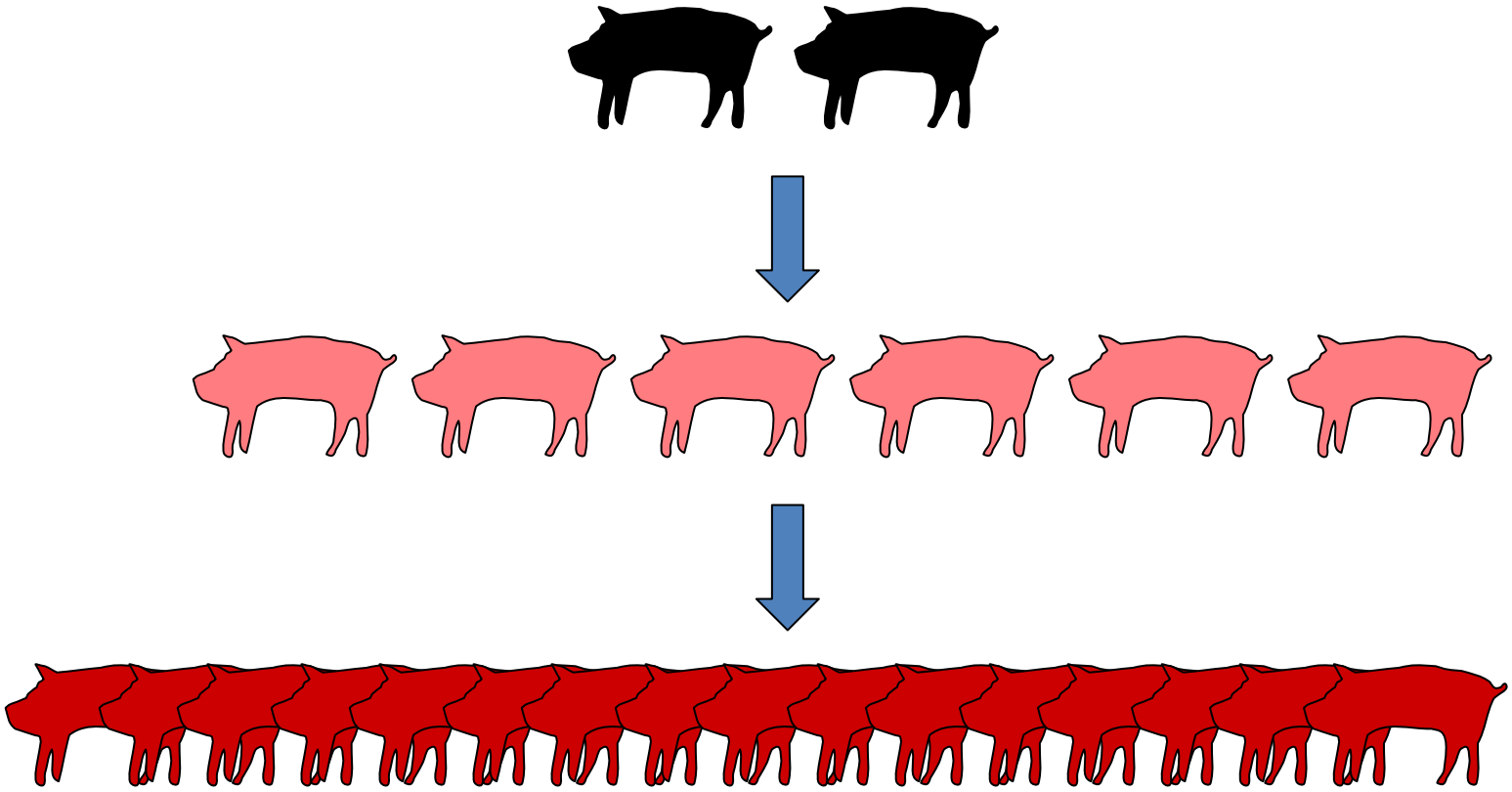
Breed reconstruction with Semen



Prolificacy influences breed reconstruction. With low prolific species, higher number of females (and semen doses) are needed.

Prolific Species

- Each female leaves multiple offspring so few commercial females are needed



Non-prolific Species

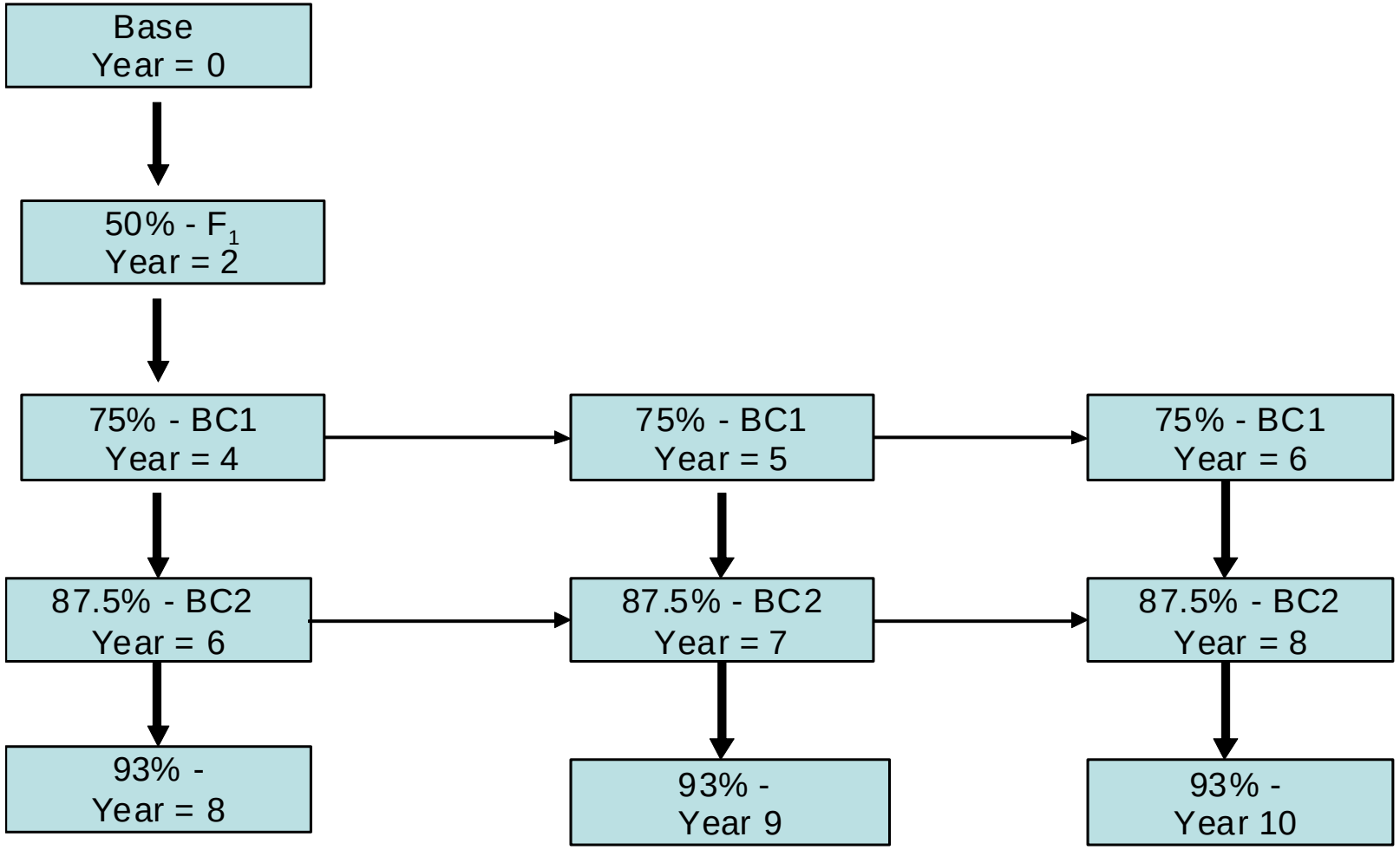
- Each female leaves few offspring so many commercial females are needed

Several thousand doses of semen may be needed!

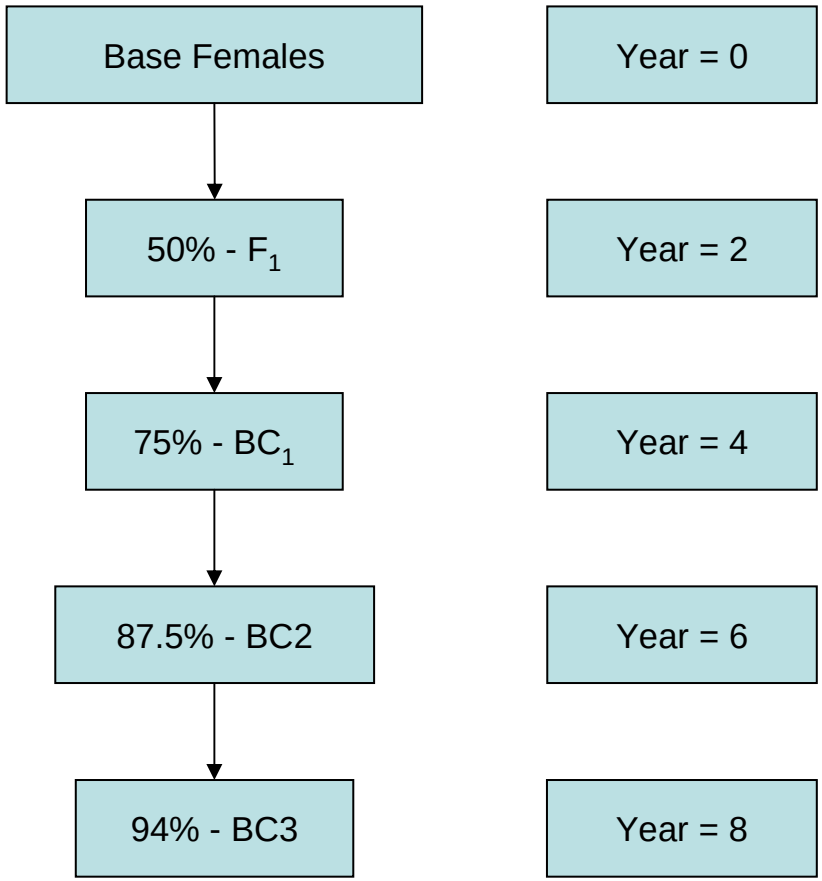
Reconstructed population

In breed reconstruction, with low prolific species, we can vary the number of parturitions per female (time of use of females). Then, time for reconstruction, and n. of semen doses and of adult females required will also change.

Breed reconstruction with semen: time of reconstruction using females from => 75% for three parturitions.



Breed reconstruction with semen: time of reconstruction using females for one parturition only (n. of semen doses and females increases)



Breed reconstruction with Semen, amount of doses

To compute the number of semen doses we must take into account:

- N. adult females to be reconstructed, and % genes of the breed to be recovered
- N. of semen doses per parturition
- N. of fertile daughters expected per female during their lifetime:
  - N. of daughters per parturition
  - Survival of daughters from birth to parturition/s
  - N. of parturitions per female (affects time for reconstruction)

Breed reconstruction with Semen, amount of doses

Semen doses needed to create 25 females = d x F x np

Where:

d = n. doses needed per parturition

F = n. of females to be inseminated = 25 x ($r + r^2 + ... + r^n$)

r = 1/(expected lifetime production of fertile daughters per female)

np = n. of parturitions per females to obtain the expected lifetime production of fertile daughters per females

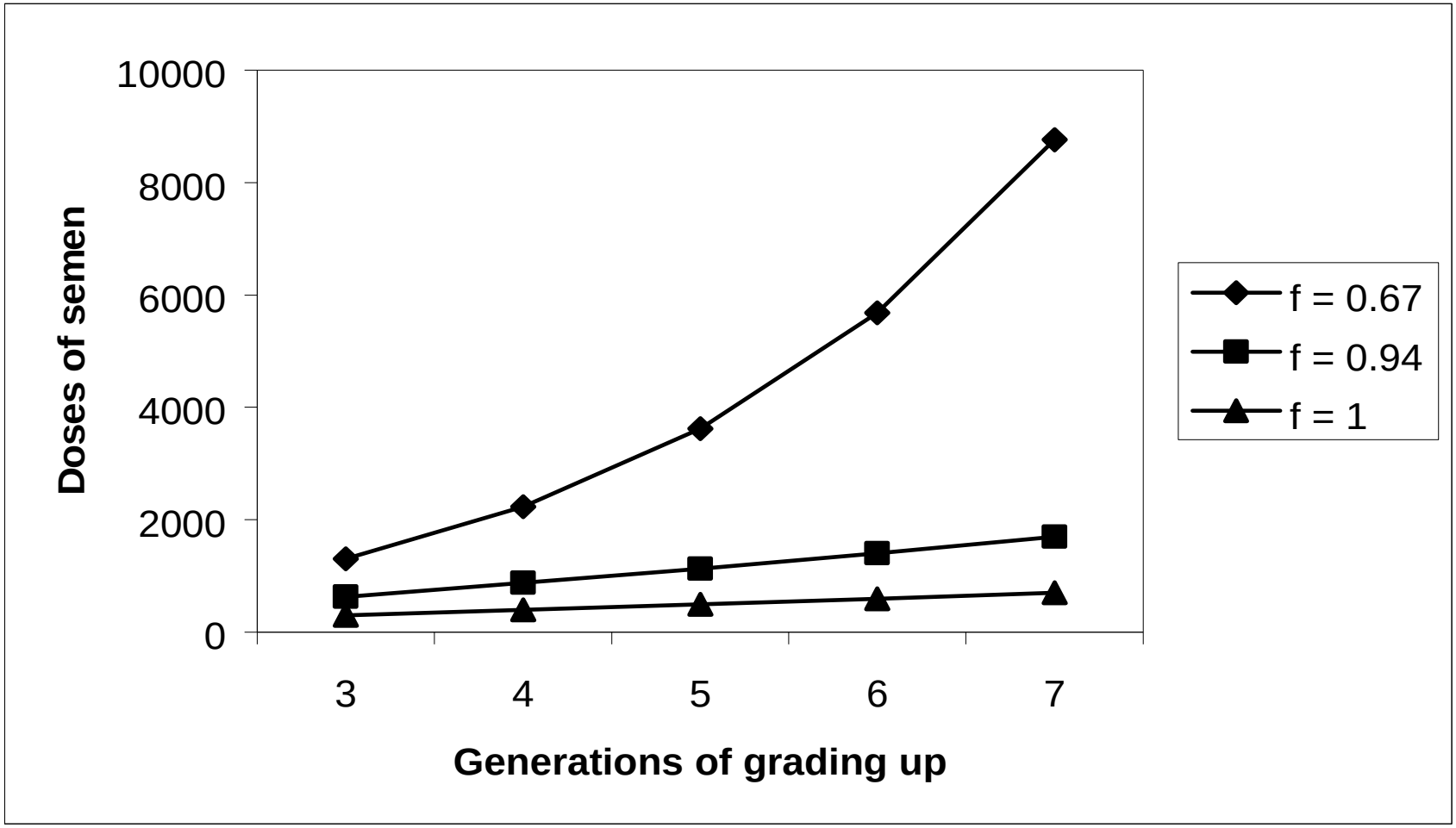
Number of donors and doses of semen for cryostorage, as a function of strategy and species.

Boettcher et al. (GSE, 2005); Gandini et. al (GSE, 2007)

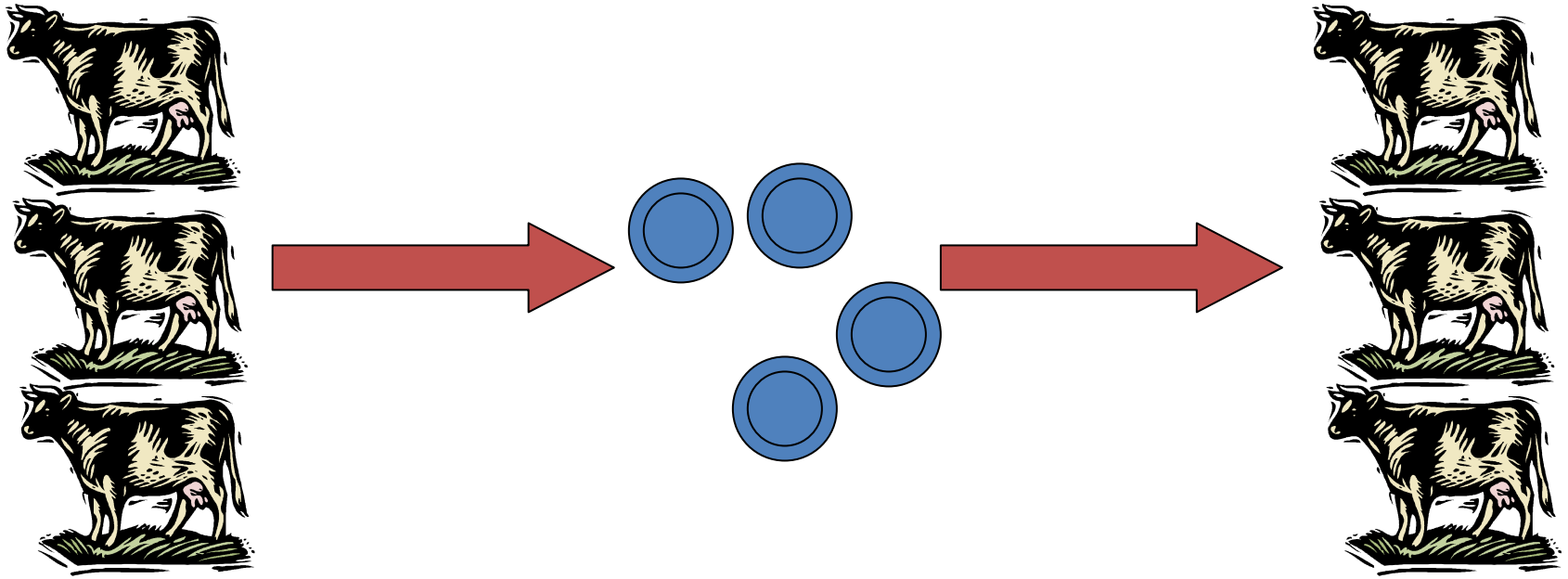
Strategy	All species	Cattle / Horse	Sheep	Pig / Rabbit	cattle-ET
	Male donors	Doses of semen			
Semen-only MAXP 4	**25**				
5		**1,172**			
4		**1,272**	**798**		
3		**1,664**	**822**		
2		**3,620**	**1,134**		**484**
1		**25,684**	**5,998**	**260**	**612**

MAXP = Maximum number of parturitions allowed during reconstruction before culling

Number of doses of semen for breed reconstruction, as a function of number of generations of grading up and lifetime production of fertile daughters by female (f).



Breed reconstruction with Embryos



Conserved population

Embryos in bank

Reconstructed population

Operational Steps – Type of material: Embryos

$$N_{emb} = 25 / (0.5 \times C \times Sr \times Se)$$

Where:

N_{ooc} = n. of embryos needed to reconstruct a population of 25 females of breeding age

0.5 = pr. to be a female

C = conception rate

Sr = pr. survival recipient

Se = pr. survival embryo from conception to breeding age

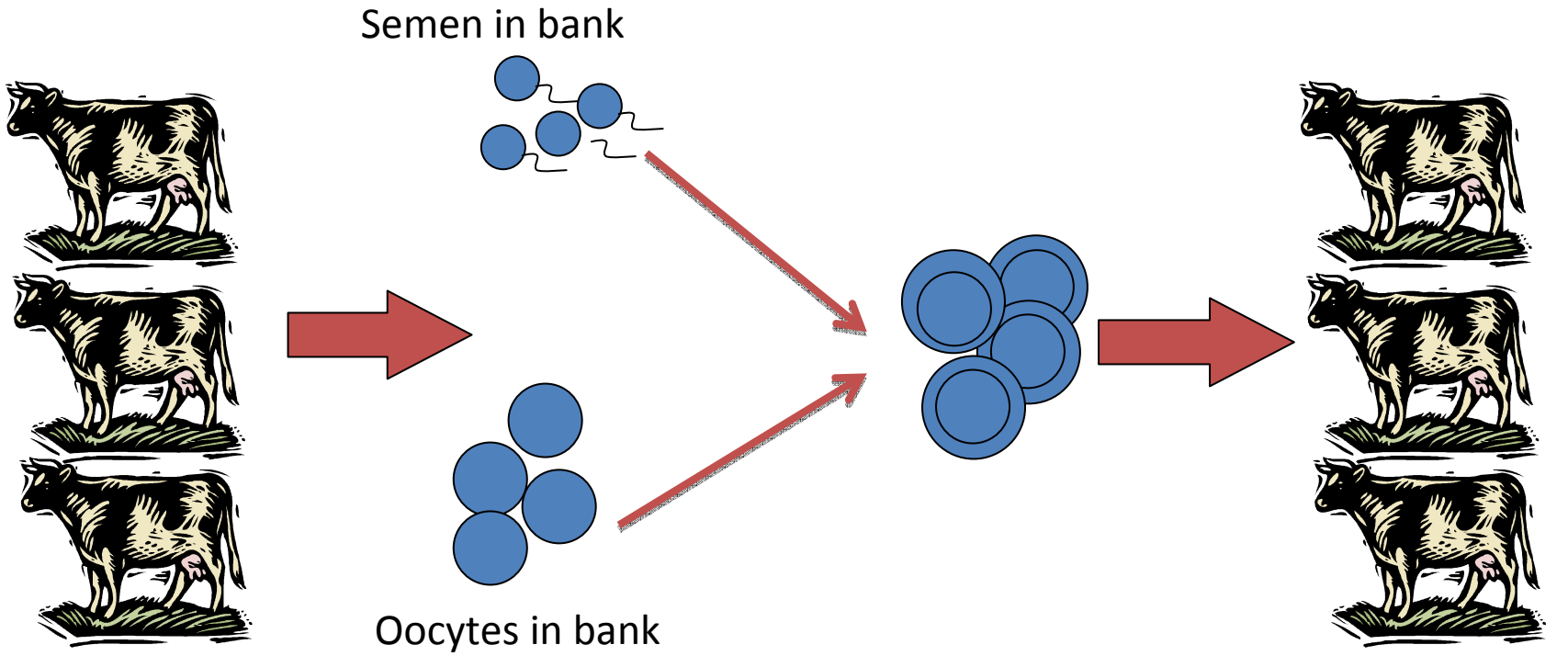
Number of donors and embryos for cryostorage

Boettcher et al. (GSE, 2005); Gandini et. al (GSE, 2007)

Strategy	All species		Cattle / Horse	Sheep	Pig / Rabbit	cattle-ET
	Female donors	Embryos				
Embryos-only	25	348 - *430*				

Expected values. In italics, 90% percentile.

Breed reconstruction with Oocytes



Operational Steps – Type of material: Oocytes

$$N_{ooc} = 25 / (0.5 \times Sivf \times C \times Sr \times Se)$$

Where:

N_{ooc} = n. of oocytes needed to reconstruct a population of 25 females of breeding age

0.5 = pr. to be a female

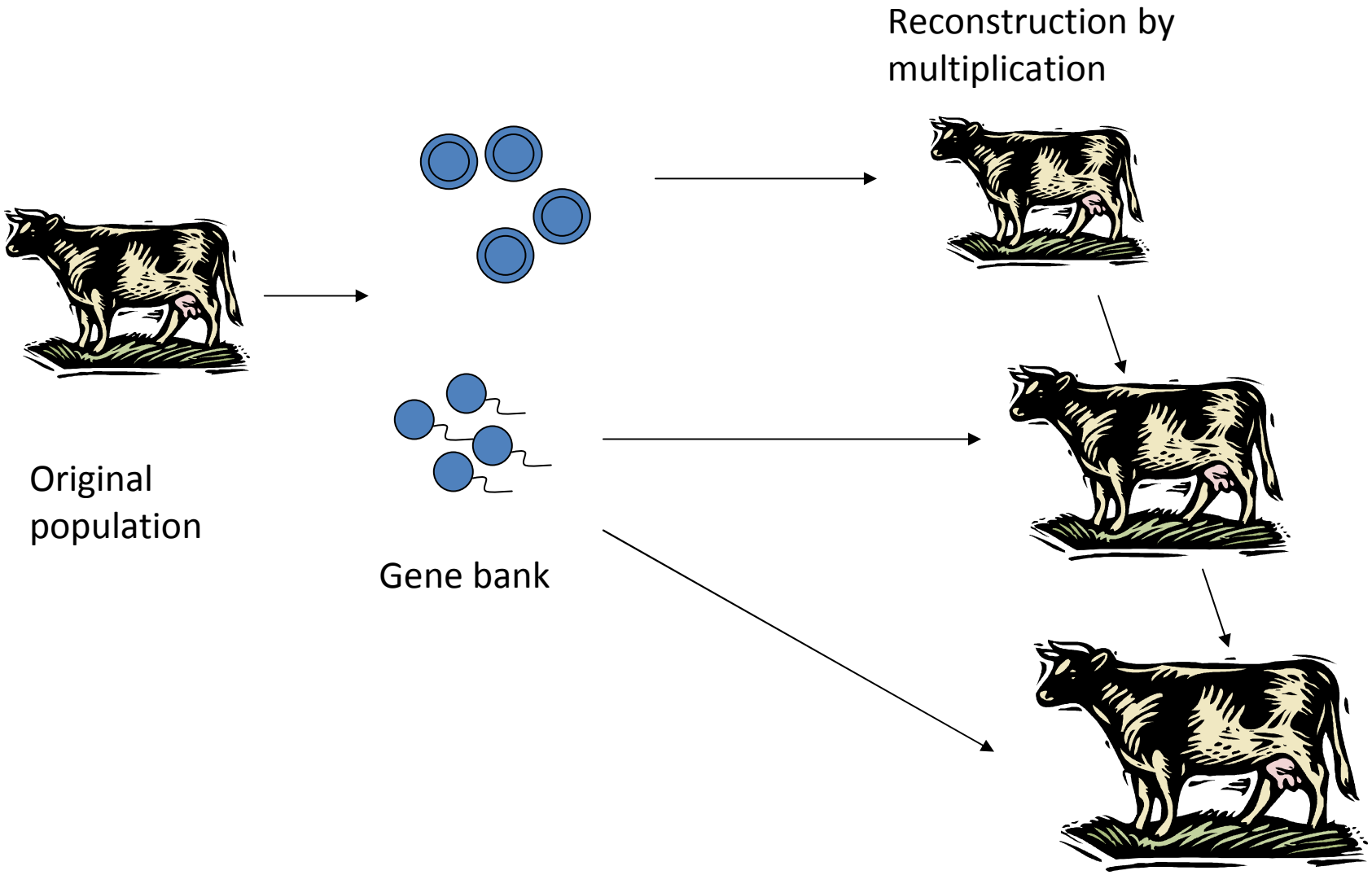
Sivf = pr. of success of the IVF procedure to obtain an embryo from oocyte (Semen doses needed)

C = conception rate

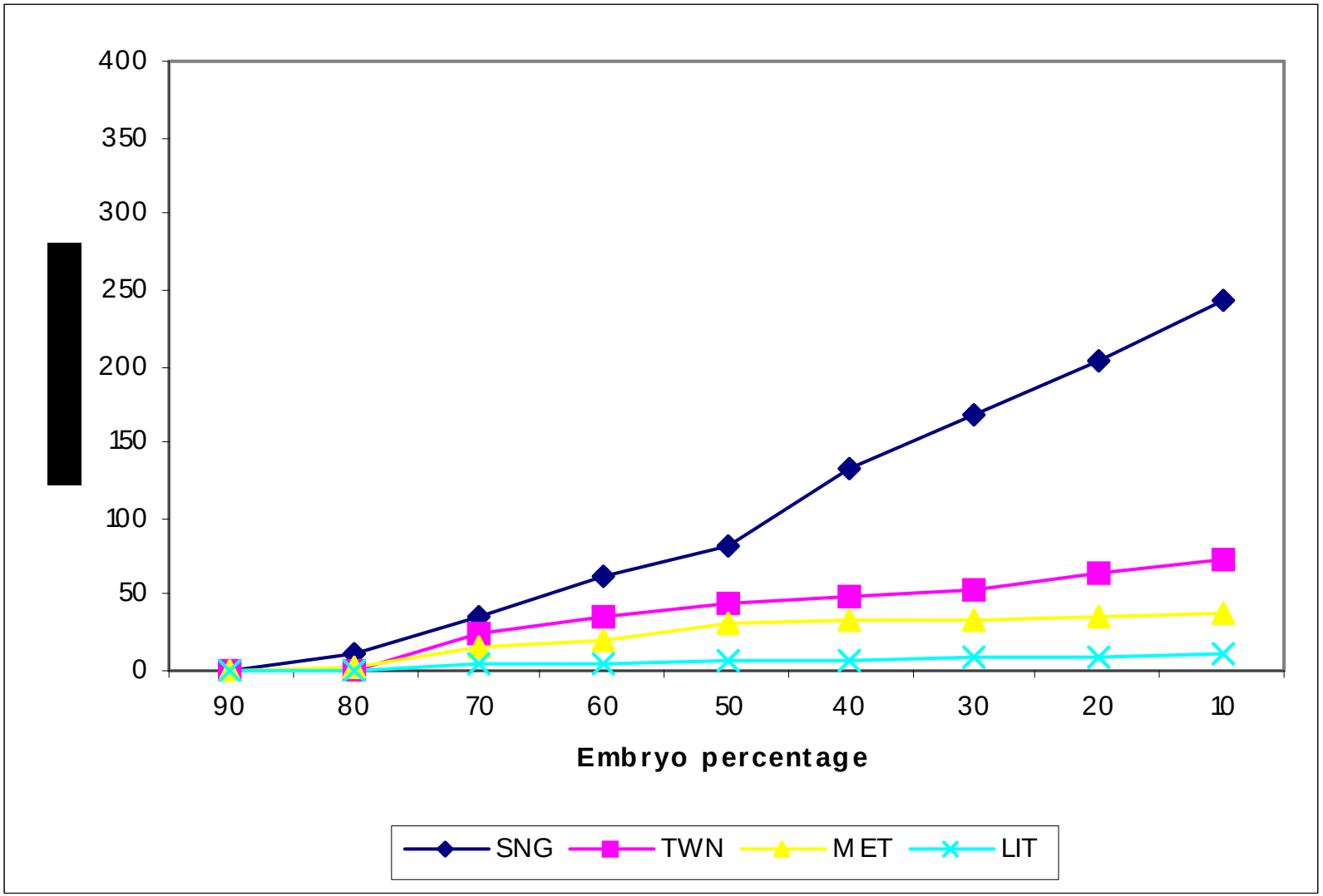
Sr = pr. survival recipient

Se = pr. survival embryo from conception to breeding age

Breed reconstruction with Embryos + Semen



Median, for four types of livestock species, of numbers of doses of semen required to reconstruct a breed when the number of embryos stored in the gene bank ranged from 90 to 10% of that required to reconstruct the population with only embryos. *Boettcher et al. (GSE, 2005); Gandini et. al (GSE, 2007)*



Number of donors, embryos, and doses of semen for cryostorage, as a function of strategy and species (duplicated for two storages)

(Boettcher et al. (GSE, 2005); Gandini et. al (GSE, 2007))

Strategy	All species			Cattle / Horse	Sheep	Pig / Rabbit	cattle-ET
	Femaledonors	Male donors	Embryos	Doses of semen			
Embryos-only	25		348 - *430*				
Embryos+semen							
% embryos 3							
90	22	3	388	22 – *96*	14 – *48*	4 – *14*	6 - *30*
80	20	5	344	50 – *150*	28 – *88*	8 – *20*	14 - *54*
70	17	8	300	88 – *184*	52 – *112*	12 – *24*	36 - *84*
60	15	10	258	126 – *248*	72 – *122*	16 – *28*	52 - *96*
50	12	13	216	216 – *408*	90 – *128*	22 – *34*	76 - *120*
40	10	15	172	274 – *472*	102 – *144*	28 – *48*	98 - *138*
30	7	18	130	370 – *612*	130 – *200*	32 – *52*	100 - *128*
20	5	20	86	452 – *682*	130 – *198*	36 – *56*	92 - *120*
10	2	23	44	512 – *740*	154 - *212*	32 - *48*	78 - *108*
Semen-only							
MAXP 4		25					
5				1,172			
4				1,272	798		
3				1,664	822		
2				3,620	1,134		484
1				25,684	5,998	260	612

Breed reconstruction: amount of genetic variation included in reconstructed population

N. Donors (founders)

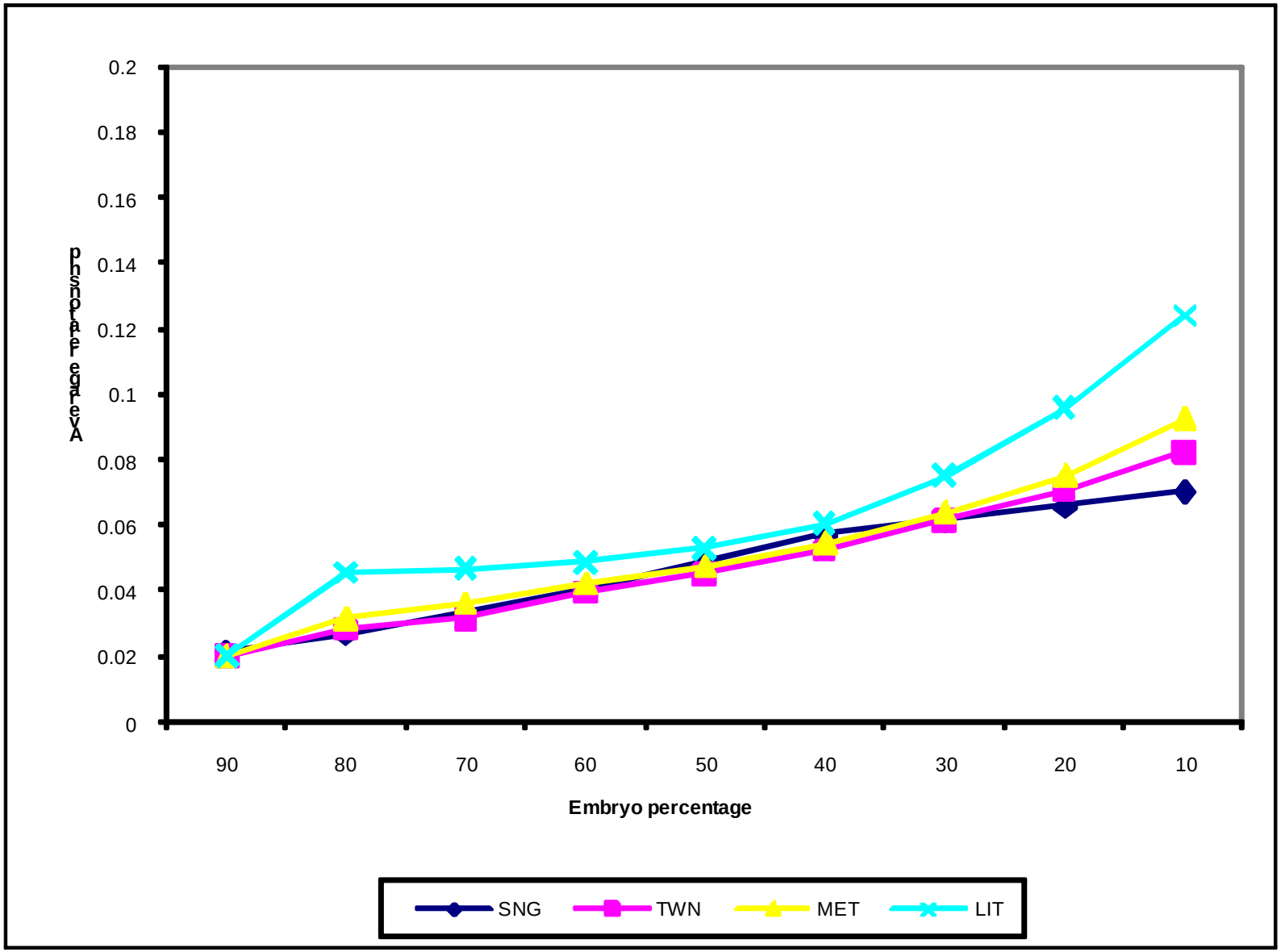
In case of random sampling, % of heterozigosity retained in the bank is $\approx 1 - (1/2N)$

where N is the number of donors. The use of 25 donors corresponds to 98% of heterozigosity retained

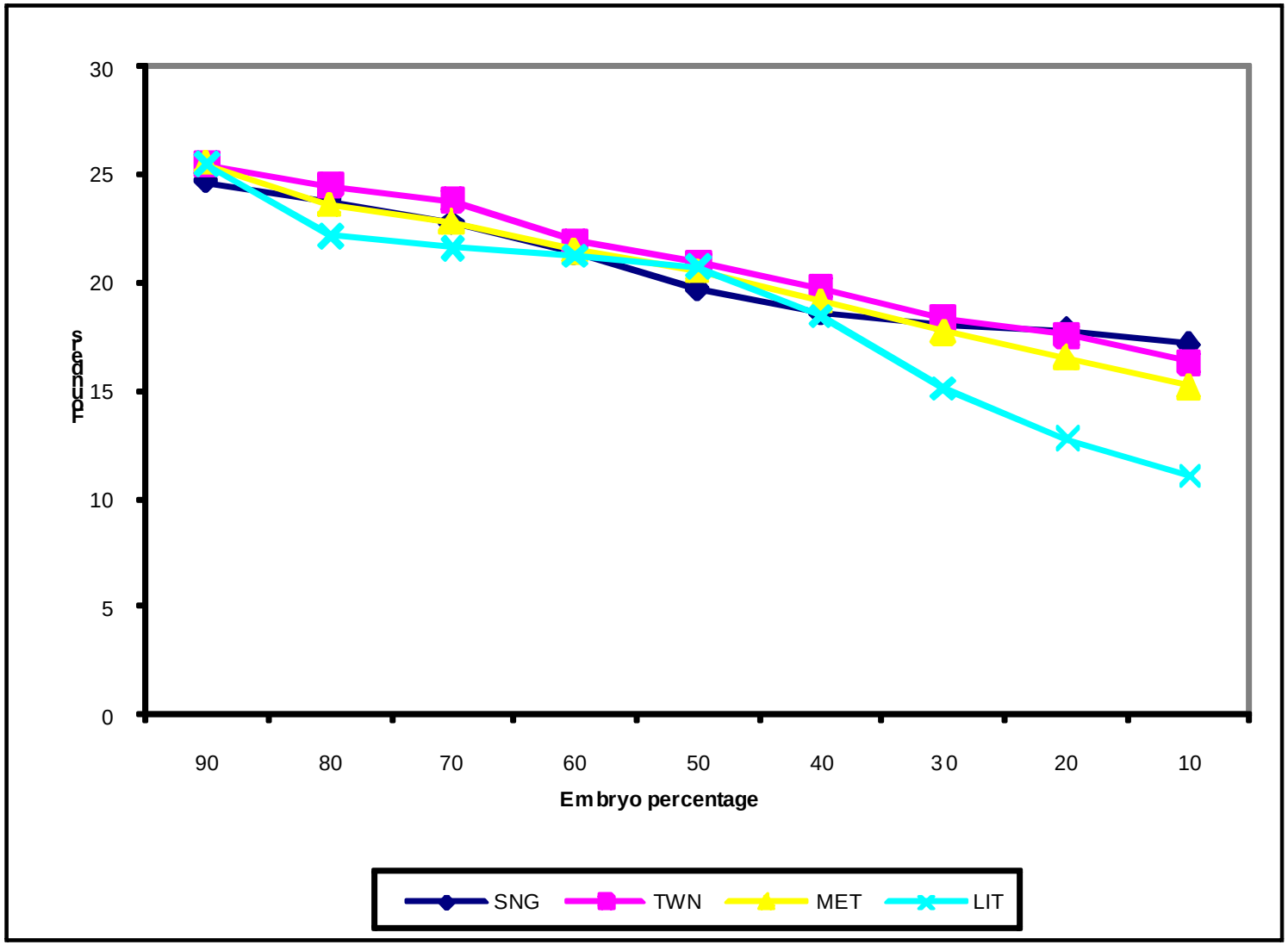
Probability of having among donors an allele with freq. p, is = $1 - (1-p)^{2N}$

Average relationship increases during breed reconstruction process. The case of embryos + semen

Boettcher et al. (GSE, 2005); Gandini et. al (GSE, 2007)

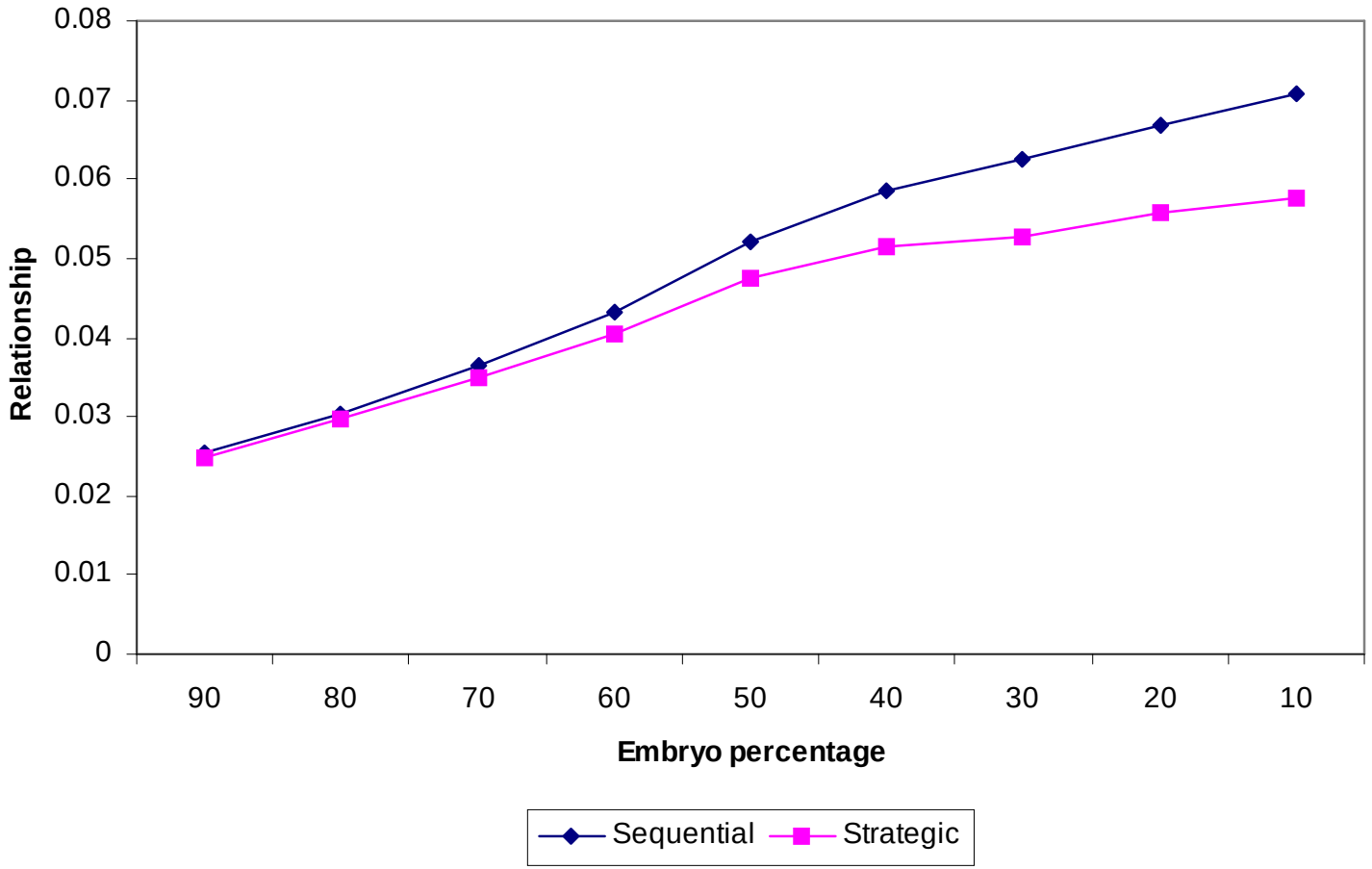


N. of founders decreases during breed reconstruction process. The case of embryos + semen. (*Boettcher et al. (GSE, 2005); Gandini et. al (GSE, 2007))*



Controlling loss of variation during reconstruction

Means (for proportions of embryos ranging from 90 to 10%) of additive genetic relationships among members of the reconstructed population of a species with single offspring when the sires are used in sequence or strategically to equalize their contribution to the final population. ***Boettcher et al. (GSE, 2005); Gandini et. al (GSE, 2007)***



Operational Steps – Frequency of collections

Frequency of collection should be considered to update the collection. To define intervals between collection (Verrier et al., 2003) **we should take into account:**

-The selection intensity of the population
-The rate of evolution of the population structure
-Risks of occurrence of genetic problems
-Costs and available funds
-Occurrence of opportunity for collection