

Cloning and embryo transfer in selection plans in horses

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This article focused only on two points about cloning which may modify selection plans: reproduction of geldings and increase of reliability of mare candidates for selection. These results have been balanced with the impact of embryo transfer.

The first advantage to be expected from the cloning is the broadcasting of the realized genetic progress. It is obvious that to have 10, 20, 30... copies of a well known champion rather than the only one would be a considerable advantage for a nation in international competition, and a much more pleasant situation for the riders. However this exploitation of an already existing superiority neither creates in itself a progress, nor generates genetic variety. The aim of this article is to consider the advantage of the cloning as generator of progress.

The use of clones may influence a breeding scheme by increasing the reliability of the candidate for the selection by multiplying the performances realized by the same genetic individual under the shape of several alive clones. For the mares, cloning also increased their reproductive capacity: the cloning of embryonic cells allows the birth of several clones per year for the same mare. Finally the somatic cloning may lead to the birth and the reproduction of clones of geldings already champions. That is the way already explored with the birth of clones of Pieraz, World Endurance Championship, and ET, Show jumping international champion, both geldings. It is the more popular way presented to encourage cloning and so it is the way we first explored too.

1 THE IMPACT OF THE BREEDING OF GELDINGS.

In that section, cloning was used to obtain one stallion able to have foals from one gelding which had already own performances. The genetic purpose is of making a breeding animal out of sterile animal. The clone was obtained from gelding after the realisation of the performance for selected geldings. We assumed that this cloning was always successful.

1.1 Material and method

The annual genetic gain was calculated with the very simple asymptotic formulae, without overlapping generation, only one selection stage. Genetic superiority was calculated only on the male way.

$$\Delta G = i_s r_s \sigma_a / (T_s + T_d)$$

with i_s =selection intensity on the sire way, r_s =reliability on the sire way, σ_a =genetic standard deviation, T_s =generation interval on the sire way, T_d =generation interval on the dam way

The structure of the population was as follows, mimicking jumping horse context. The number of progeny by sire was 40; fertility of mares was 55%. The sex ratio of born foals was 1/2. The reproductive life of a stallion was 10 years. Performances were realised at 5 years old, and 50% of the births had a performance. Heritability was 0.30. The selection criterion was a genetic evaluation including performance of the horse itself, his parents, half sibs from the same sire and progeny. So that when we speak about selection on own performance, it means selection on genetic evaluation including own performance *and* performance on relatives mentioned above. The number of relatives used for this

evaluation depended on the strategy of selection which determined the age of the male and relatives when he was selected. Generation interval on the female way was fixed at 11 years.

Even in a population where only the geldings realize performances rather than males for technical or practical reasons, there are other alternatives that the cloning to realize a selection. We can imagine a selection by siblings ($\frac{1}{2}$ sisters and $\frac{1}{2}$ gelded brothers) or by progeny. In any case, genetic evaluation used for selection was done from all relatives but the decision of selection was performed at different time according to information available. That is why I compared the various strategies of selection:

- Reference : selection from own performance (when males can have performances). In that case, age at selection was the end of the 5th years, after performance, and so age at first progeny was 7.
- Selection from sibling. In that case, age at selection was 2 years old, as own performance is not necessary and so age at first progeny was 4.
- Selection by progeny. In that case, 36% of sires in activity were used for progeny test, so without any genetic superiority. For selected stallions, age at selection was 9, due to the time spent by progeny to reach 5 years old if tested stallions had their first progeny at 4 and age at first progeny after selection was 11.
- Selection by cloning of a gelding with own performances. In that case, selection occurred when geldings were aged 5 but 3 years more were necessary for the clone to be born and to become his reproductive life and so age at first progeny of the considered gelding was 10.

In these cases, excepted for the first one which is the reference, males do not participate in a competition. In the reality, a proportion of the horses with performance is male, another proportion is geldings and the choice of the breeders can be made among males and among cloned geldings. So, genetic progress was also calculated with various proportions of males with performances (from 100% to 0% by 10%) and with various proportions of breeders taken from males or clones of gelding (from 0% to 100% by 4%, i.e. for 25 stallions from 0/25 to 25/25 by 1/25).

1.2 Results

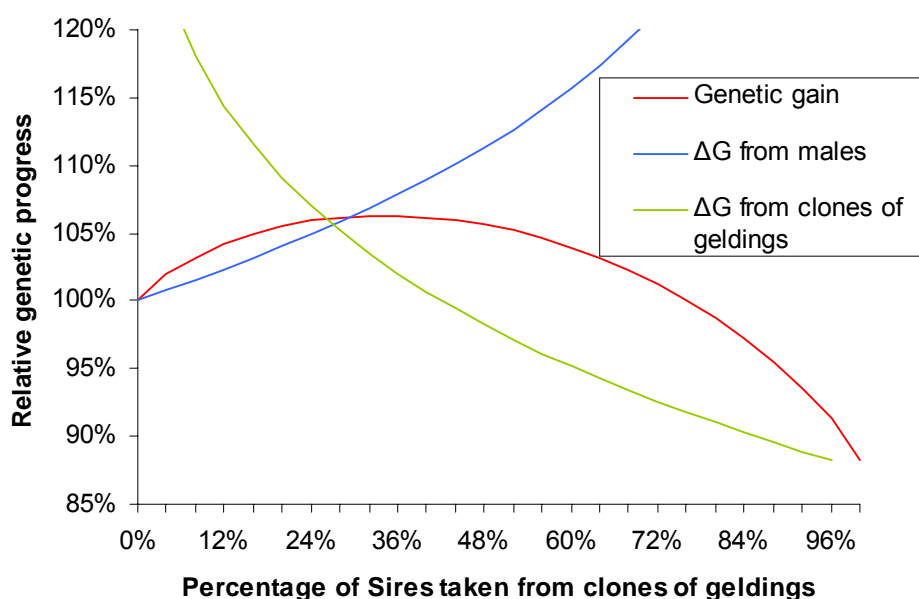
Table 1 showed results of the 4 strategies of selection.

Table 1 – Various selection strategies to supply lack of performance on males.

Selection strategy	% foals born from selected stallions	i_s	r_s	T_s	ΔG
Reference	100%	2.456	0.657	11.5	0.07168
Sibling	100%	2.575	0.482	9.5	0.06061
Progeny	64%	1.465	0.744	15.5	0.02633
Cloning	100%	2.456	0.657	14.5	0.06324

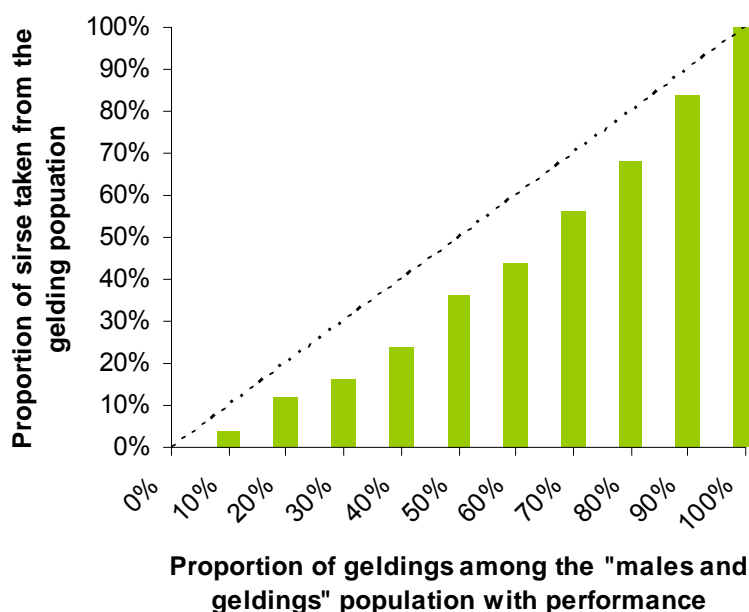
The figure 1 was calculated in the case of 50% of geldings among “males and geldings” in the population with performance. The figure illustrated genetic progress realised according to the proportion of sires taken from the clones of gelding population (from 0% to 100%). The other sires were taken from the male population. The genetic progress showed a maximum for 36% of sires chosen among geldings which were cloned. This maximum is due to the decrease of genetic progress obtained from selection of geldings and the increase of genetic progress obtained from selection of males when the percentage of sires taken from geldings increased. This is due to the evolution of selection intensity.

Figure 1 – Relative genetic progress in the case of 50% of males and 50% of geldings in the population with performance, according to various proportion of breeders taken from males with own performance or clone of gelding with own performance. The reference was selection on males only without cloning.



The figure 2 gave the proportion of sires taken from geldings at the maximum of genetic gain in each case of proportion of geldings among “sires and geldings” population with performance. This proportions were not equals: less geldings were taken to become sires (after cloning) than available in the population with performance than males.

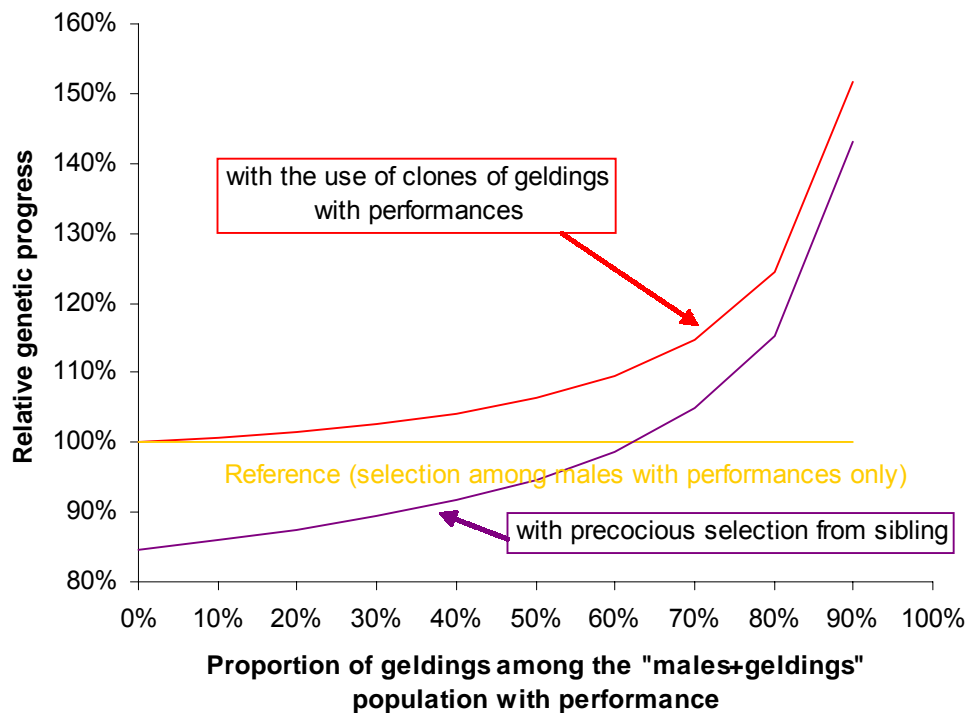
Figure 2 – Proportion of sires from gelding population after cloning according to the proportion of geldings with performances.



The figure 3 was computed for different proportion of gelding among the “males and gelding” population with performances, in each case at the maximum of genetic gain obtained according to the

percentage of sires taken among males or geldings. The figure showed a significant increase when the proportion of geldings in the population with performance is higher than 40%.

Figure 3. Relative genetic progress of selection with the help of clones of geldings or selection from siblings compared to selection among males only according to the proportion of geldings among the population with performances.



1.3 Discussion

When comparing cloning of geldings with other strategies of selection when performances for males were not available, the advantage of cloning is only 4% of genetic gain (0.6324 versus 0.6061) better than selection on siblings (with genetic evaluation). In that case, genetic gain is only 8% less than if performances were available for males and selection was performed on own performance with a genetic evaluation with relatives. So advantage of cloning is not very large but few percent of genetic gain may be important in a strategic view point in a production where extreme horses have exponential values. More, selection on siblings requires the recording of the performances and genealogies while the cloning can be directly made on the exceptional horses without heavy collection of performance management (but not necessarily less expensive).

When selection is on own performance with some males and some geldings having performance, one must take care to select the good proportion of sires from males and geldings depending on the percentage of geldings with own performance. The relation between percentage of sires taken from clones of gelding rather than males and percentage of gelding having performances among all males and geldings is not linear. The optimum was for example 24% of sires from clones of geldings when 40% of the “male and gelding” population with performance are geldings. So cloning is limited to really the best gelding horses, selection is more drastic than for males. In that case the use of clones become to be really significant, comparing to selection only among male population, when the percentage of gelding is higher than 40% in the male and gelding population. A contrario, when this percentage increase, the relative progress obtained from selection from sibling was more and more competitive: advantage of cloning comparing to sibling selection decreased from 14% to the 4% previously mentioned. The percentage of geldings in the “geldings and males” population with performances is supposed to be at random in the population, male horses are not the best one but only a random

sample of the population. So it is difficult to compare this percentage with true percentage in competition as jumping or dressage because very often, only best horses were not castrated. In order to have an idea of the range of this percentage in jumping, we selected Selle Français horses born from 1981 with performance in jumping before 2001. To mimic selection of sires, we extracted the 2% best horses, males or geldings, of this population, based on regular genetic evaluation (BLUP animal model). In these 2%, the percentage of gelding was stable, between 50% and 76%, from birth year 1981 to 1988 but then decrease to reach 1/3 in 1990/1991 and only 15% in 1995. So it seems possible to have in jumping a high percentage of male in competition but cloning may be an opportunity to reduce this percentage which should be easier for riders. In other discipline as endurance races, the effect of the sex is unfavourable for males in competition, and it could be a true opportunity. At present (2002-2005), the percentage of geldings from male and gelding population with result in endurance races was 70%. In that case, if breeders do not want to select very young horses from relatives (2 years old) or in the lack of information on these relatives, cloning of best geldings with performance should improve genetic gain of 15%.

2 THE IMPACT OF INCREASE OF RELIABILITY DUE TO CLONING OF EMBRYO ON THE FEMALE WAY.

In that case, cloning is assumed to make possible the birth of 5 genetically identical foals by year from one mare by cloning of a single embryo.

2.1 Material and methods

The annual genetic gain was also calculated with the very simple asymptotic formulae, without overlapping generation, only one selection stage. Genetic superiority was calculated only on the female way.

$$, \Delta G_d = i_d r_d \sigma_a / (T_s + T_d)$$

with i_d =selection intensity on the dam way, r_d =reliability on the dam way, σ_a =genetic standard deviation, T_s =generation interval on the sire way, T_d =generation interval on the dam way

The structure of the population was slightly different than in the first example but the aim was the same. The fertility of females was 59%, the reproductive life of mares was 12 years. Performances were realised at 5 years old, and 50% of the births had a performance. Mares were selected at 5 years old among horses with performances. Heritability of the trait was 0.27. The selection criterion was the own performance.

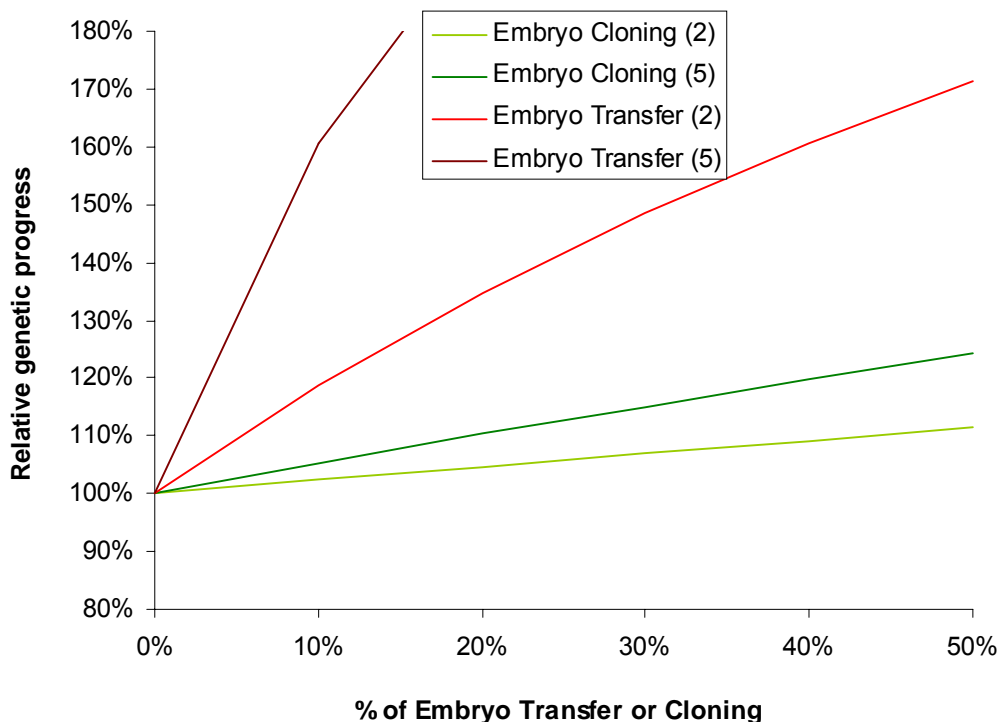
The selection scheme with embryo cloning enabled to have less reproductive mares genetically different to produce embryos (other mares were only used to be surrogate mother). But, as the number of horses with performance is a constant due to the sport market, the number of candidates genetically different with a performance is also reduced in the same proportions. So selection intensity was the same. But reliability of candidates for selection was different. In that case, a candidate with 4 more clones in competition should be assumed to have 5 performances instead of 1 and with a repeatability close to heritability because in that case, difference between repeatability and heritability is only due non additive genetic component but not common environmental effect as clones were dispatched in different places. Finally, model was done with repeatability equal to 0.35.

This model was compared to the influence of embryo transfer without cloning. In that case the number of mares needed to produce next generation was lower than in the reference scheme, identical to scheme with cloning. But the number of candidates genetically different with performance was higher, as the different embryo were only half sibs and not genetically identical. In that model, selection intensity was computed without taking into account reduction of variance due to relationship between candidates.

2.2 Results

In the standard scheme, selection rate was 70%, hence selection intensity was 0.497. Reliability was 0.5225. Generation interval was 13 years. With cloning, only reliability changed according to the percentage of embryos cloned. With embryo transfer (TE), the number of mares needed each year was divided by $(\% \text{ of mares with TE} \times \text{number of embryo transferred} + (100\% - \% \text{TE}))$. So that selection intensity increased according to the percentage of TE. The figure 4 presented the genetic progress according to percentage of mares subject to cloning or embryo transfer and the number of clones or embryo per mare.

Figure 4 – Relative genetic progress with embryo cloning or embryo transfer on the female way. In (), the number of transfers by mare.



2.3 Discussion

As cloning only increased reliability the gain in genetic progress was low. For 10% of mares subject to embryo cloning (140 mares), which is already far away from what is at present technically possible, the increase of genetic progress is only 2.4% with 2 clones for one embryo and 5.3% for 5 clones. For the female way, the technique of embryo transfer itself is more encouraging. As it increased the capacity of reproduction, it increased the selection intensity which was the weakness of the female path in the genetic progress. For 10% of mares subject to embryo transfer, the increase was 18.8% for 2 embryo per mare and 61% (!) for 5 embryos per mare. The progress may be more important if only best mares were chosen for embryo transfer (which is the case in practice). At present 1.7% of Selle Français mares have embryo transfer with mean 1.5 embryos by mare.

3 CONCLUSION

This small analysis of some possibilities of cloning for improvement of genetic progress gave only some ideas. More work must be done with very different scheme, for example with focus on mare of sires which is perhaps the most critical point of our kind of selection. The purpose here was principally to discuss about the opportunity of somatic cloning for geldings to make them able to have progeny. This cloning is interesting in population where a high percentage of geldings are competing ($\geq 40\%$) because of advantage of geldings for competition (management or performance). In that case the increase of selection intensity due to a larger number of putative candidates offset loss due to higher

generation interval due to waiting the sexual maturity of the clone. The expected gain for 70% of geldings in the population with performance was 15%. This result should be confirmed by a correct analysis including overlapping generation and survival curves. For the female way the most promising way is embryo transfer in order to increase selection intensity while reducing the number of mares for producing the next generation. With 10% of mares with embryo transfer and 2 embryos by mare, the genetic gain is 19%. This result should also be confirmed and was probably underestimated is this embryo transfer is reserved to best mare, susceptible to become mare of stallions. In any case, prospect was made with technical solutions still far away from what available today. More, the economic cost of such solutions should be discussed in regard of genetic gain expected, and compared to other simpler improvement which may be made at lower cost.

ABSTRACT

Genetic trend was calculated from $i r \sigma_a / T$ with i =selection intensity, r =reliability, T = generation interval σ_a genetic standard deviation in different situations involving cloning and embryo transfer. The first situation was use of somatic cloning of geldings with own performances to become stallions when they were 3. In current sport situation, comparing to selection among males only, the genetic progress increased +4% with 40% of geldings among horses with performances. With 90% of geldings among horses with performances, cloning increased genetic progress 52% compared to selection on males with performances but only 6% comparing to selection of young males aged 3 on parental information (females and geldings as half sibs), before gelding. The second situation was the use of cloning of embryo as multiplication of own performances for the horse subject to selection. In the male way, the increase of genetic progress was very low, about 1% with 5 horses per clone and 40% of candidates with clones in competition (which supposed a very high technology). On the female way, the increase of reliability on candidates due to the existence of clones is also following by an increase of number of progeny per female. The late increase may also be obtained by embryo transfer (ET). The genetic progress increased 98% with 20% of female with ET among whole population and only 10% with cloning only but 204% with cloning and ET.