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Overlooking for new lines of rabbits synthesized from genetic improvement programs achieved in Egypt and Saudi Arabia

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ABSTRACT

Few numbers of new lines were synthesized in our Arabian hot climate countries using different criteria and methods of selection and crossbreeding particularly in Egypt and Saudi Arabia. In this concept, Saudi 2 and APRI rabbits (as maternal lines) and Alexandria and Saudi 3 (as paternal lines) and Moshtohor (as multipurpose line) were being formed to be convenient in hot climate. The most common selection criteria used in selection programs to develop new maternal lines were related with litter size at birth or at weaning and milk production, while in paternal lines, post-weaning daily gain or marketing weight are commonly selected individually. Spanish V-line rabbits genetically selected for more than 35 generations were introduced in various developing countries (as alive animals or as frozen embryos) and by using recent bio-techniques and applying selection and /or crossbreeding programs with local lines, this line was widely distributed in some hot countries of the world like Egypt and Saudi Arabia. Direct selection had little or moderate effects on litter and lactation traits, while it had considerable effects on post weaning growth and feed conversion. Direct heterotic effects were evidenced for litter size, litter weight, and milk yield. Crossbred does and dams gave favourable heterotic effects on litter traits, milk yields and components and milk conversion ratio. Direct heterosis for body weights raised in hot countries were mainly positive and ranging from 1.3 to 14.5 %, but the estimates for maternal heterosis were mainly low and ranging from 0.2 to 5.3 %. Crossbred dams gave moderate maternal heterotic effects ranging from 4.8 to 18.7%. Neither individual heterosis, nor maternal heterosis were evidenced for meat quality traits. Crossbred bucks were associated with an existence of heterotic effects in ejaculate volume (11.6%), sperm concentration (10.5 %), percentages of motile (9.8%) and living sperms, along with a reduction in percentages of abnormal (-10.8%) and dead sperms (-23.5%). Crossbred dams gave maternal heterotic effects on some semen parameters in their progeny of crossbred bucks. The recent molecular technologies were used only in developing countries to detect the associations between phenotypic traits and genetic markers and three markers were detected for litter and milk traits and body weights.

Key words: Rabbits, genetic improvement, selection, crossbreeding, synthetic lines.

INTRODUCTION

In our Arabian hot climate countries, little numbers of synthetic maternal and paternal lines of rabbits were synthesized using different criteria and methods of selection and crossbreeding (Al-Seaf et al, 2008). Also, long-term selection experiments carried out in rabbits for more than 10 generations were few (El-Raffa, 2000; Khalil *et al.*, 2005; Youssef et al, 2008; Iraqi et al, 2009). Nowadays, synthetic lines are being formed in Egypt and Saudi Arabia by crossbreeding and selection for defined objectives (El-Raffa, 2007; Khalil and Al-Saef 2008; Youssef et al, 2008; Iraqi et al, 2009). Some attempts to develop new lines already done and **V**-line has proved to be advantageous to standard Californian and New Zealand White for litter size traits and daily gain (Iraqi et al, 2006). Recently in Egypt, new synthetic lines of rabbits called Moshtohor and APRI as maternal lines and Alexandria as a paternal line were being formed to be convenient in hot climate. Till now, multi-purpose synthetic lines were not formed to be used on small and large scales. Three-way crossing will be beneficial in this concept. This scheme has the advantage of exploiting at each generation the entirety of the effect of heterosis (direct and maternal), but it requires a complex scheme, based on the maintenance and selection of the pure stocks and the multiplication and diffusion of the crossbred bucks and does. This program makes possible to provide farmers with improved animals, while ensuring their independence.

In practice, the following programs of genetic improvement were carried out in Europe and USA to achieve (Baselga, 2004):

(1) Producing specialized strains that are selected to produce F1 females, which are crossed with a sire of another strain to produce terminal product. This method has the advantage of exploiting at each generation the entirety of the effect of heterosis (direct and maternal), but it requires a complex scheme, based on the maintenance and selection of the pure stocks and the multiplication and diffusion of the crossbred females. It is the reason why these turnkey programs are rarely used in developing countries, except in some small or large scale projects in Egypt and Saudi Arabia. They need an important investment and create a technical and economical dependence which does not usually fit the social and economical environment.

(2) Creating a synthetic line by crossing females of a local population or breed (well adapted to the environment) with imported males or semen from a selected strain to produce F1 population; it will be bred without selection during few generations, avoiding consanguinity, and constitute a nucleus submitted to selection. This program makes possible to provide farmers with improved animals, while ensuring their independence. It lets to the farmers to adapt their strategy of renewal of their herd and they can practise self-replacement without loss of the genetic level, buying males or the two sexes to the nucleus.

(3) Selection within pure breeds (local or imported). This program is less used, for different reasons: local populations performances are usually very low, even if they are well adapted and imported breeds often do not adapt to poor environments and, in both cases, heterosis are not completely exploited.

The main objectives of this article are concentrated in evaluating sustainable genetic improvement programs in Egypt and Saudi Arabia in terms of: (1) programs of selection and crossbreeding used to develop new synthetic lines; (2) estimates of selection responses obtained; (3) direct and maternal heterotic improvements obtained from crossbreeding; (4) Molecular analyses and attempts to identify the specific genetic markers.

Genetic improvement programs achieved:

Summaries for lines developed in Egypt and Saudi Arabia through crossbreeding and selection are presented in Tables 1, 2 and 3.

In Saudi Arabia, a national project of rabbit production was established to detect the possibilities of producing meat rabbits under industrialized and hot conditions (Khalil et al, 2002, 2005). For this reason, special emphases were paid to construct a genetic improvement programme to develop new lines of meat rabbits convenient for this hot country. Accordingly, V-line rabbits were imported in 2000 from Spain and were crossed with desert Saudi rabbits (Gabali, G). This program was based on an evidences stating that V-line rabbits and their crosses could produce efficiently under hot climatic conditions (Khalil et al. 2002; Al-Sobayil and Khalil 2002). From this program, two synthetic lines (Saudi-2 as a maternal line with the structure of $((¾V¼S)^2)^2$ and Saudi-3 as a paternal line with the structure of $((¾S¼V)^2)^2$) were developed from crossing Saudi Gabali with V-line rabbits, both selected for litter weight at weaning and individual weight at 84 d (Table 1). Details concerning the development of these new lines were presented by Khalil *et al.* (2002, 2005) and Al-Saef *et al.* (2008).

In Egypt, great efforts have been made since 1998 to select for one exotic maternal line under local conditions and to develop and select local lines based partially on local breeds. An Egyptian-Spanish programme was established involving Alexandria University, Animal Production Research Institute (APRI, Cairo) and Benha University. V-line rabbits were imported in 1998 from Spain and various selection experiments were practiced. The first line was developed from crossing Baladi Red with V-line and this maternal line named APRI and it was selected for litter weight at weaning (Youssef *et al*, 2008; Table 1). A synthetic paternal line named Alexandria was originated in Alexandria University from crossing V line with Baladi Black and selection was practiced for daily weight gain during 28-63 days of age (El-Raffa, 2007; Table 2). In March 2003, a selection program was started to produce a synthetic multi-purpose line named Moshtohor resulting from crossing Sinai Gabali with V-line and selection was practiced for litter weight at weaning and live weight at 56 days (Iraqi *et al.,* 2007, 2008; Table 3).

Selection responses:

In selection experiments carried out in developing countries, definite methodologies have been proposed to estimate selection responses. One of them is based on regressing the estimates of the breeding values on generations and this approach depends on the genetic parameters and the model used (Moura *et al.,* 2001). The other methodologies are not depend on the genetic parameters and the model itself but they are dependent on another approach through using the control population which could be an unselected population (Khalil *et al.,* 2002, 2005), or using the population selected divergently (Moura *et al.,* 1997).

Table 1: Programs used to create synthetic maternal lines

Synthetic line and origin	Founder breeds	Selection criteria	Selection methodology	Number of generations	Selection response per generation
Saudi-2, Saudi Arabia (Khalil *et al*, 2005)	V line and Saudi Gabali	LWW + W12	BLUP animal-repeatability model	11	LSB= 0.18 kit/litter; LSW= 0.16 kit/litter; LWW= 62g/litter; WW= 8.6g/kit
APRI, Egypt. (Youssef et al, 2008; El-Raffa, 2007)	V line and Baladi Red	LWW	BLUP animal-repeatability model	7	

LSB = litter size at birth; LSW = litter size at weaning; LWW= litter weight at weaning; WW = weaning weight; W12: weight at 12 weeks**.**

Table 2: Programs used to create synthetic paternal lines

Synthetic line and origin	Founder breeds	Selection criteria	Selection methodology	Number of generations	Selection response per generation
Alexandria, Egypt (El-Raffa, 2007)	Line V and Baladi Black	ADG (28-63d)	Individual selection using BLUP	7	
Saudi-3, Saudi Arabia (Khalil et al 2002, 2005)	Line V and Saudi Gabali	LWW + W12	Individual selection using BLUP	10	W12= 38g; ADG= 0.6g; LSB= 0.14 kit/litter; LSW= 0.12 kit/litter; LWW= 35g/litter

LSB = litter size at birth; LSW = litter size at weaning; LWW= litter weight at weaning; ADG= average daily gain.

Table 3: Programs used to create synthetic multi-purpose paternal line

Synthetic line and origin	**Founder breeds**	**Selection criteria**	**Selection methodology**
Moshtohor, Egypt (Iraqi et al, 2008)	Sinai Gabali and line V	LWW+ 56-d weight	Two-stage selection using BLUP

LSB = litter size at birth; LWW= litter weight at weaning; ADG= average daily gain**.**

As presented in Table 1, genetic responses obtained from long-term selection experiments for litter size and other litter traits were found to be moderately considerable. Selection experiments for growth rate notifying successful responses in most experiments carried out in Brazil (Moura *et al.,* 1997). Khalil and Al-Saef (2008) stated that does selected for litter size at weaning presented significant responses in feed intake (3%) and milk yield (6%). A response of 62 g per litter was recorded when selecting for litter weight at weaning. Estimates of direct selection responses per generation were moderate and ranged from 8.7 to 12.6 g for weaning weight, and 18 to 68 g for marketing weight (Moura *et al.,* 1997; Khalil *et al.,* 2005). Selection for growth rate has little or somewhat moderate effects on carcass characteristics and meat quality when the rabbits were selected at the same stage of maturity. Selection for litter weight at weaning achieved considerable responses in growth rate with maintaining high litter components and feed conversion.

Sustainable direct and maternal heterotic improvements

Different crossbreeding experiments carried out in Egypt (e.g. Khalil et al, 1995; Khalil and Afifi, 2000; Abd El-Aziz *et al,* 2002; Iraqi et al, 2007; Youssef et al, 2009) indicated that direct heterotic effects were evidenced for litter size, litter weight, and milk yield in most of the possible crossbred does obtained. Consequently, both producers and processors in this area could potentially benefit economically through using crossbred does. Also, estimates of maternal heterosis were favourable and indicating that crossbred dams had considerable maternal heterotic effects in terms of larger litter size, heavier litter weight at birth and weaning, favourable feed conversion ratio, and efficient milk to litter gain conversion ratio than their crossbred daughters (Khalil et al, 2005), *i.e.* crossbred does and dams gave favourable heterotic effects on litter traits, milk yields and components and milk conversion ratio.

For postweaning growth, estimates of direct heterosis for body weights raised in hot countries were mainly positive and ranging from 1.3 to 14.5 %, but the estimates for maternal heterosis were mainly low and ranging from 0.2 to 5.3 %, i.e. crossbred dams had little heterotic maternity over their purebred dams in these growth traits. Abdel-Ghany et al (2000a,b) and Afifi *et al*. (1994) for crossing New Zealand White with Baladi Black or Baladi Red in Egypt found that heterosis percentages ranged from 2.7 to 9.5% for post-weaning body weights and gains.

For carcass traits, Afifi et al. (1994) found that direct heterosis percentages from crossing New Zealand White X Baladi Red in Egypt ranged from 1.0 to 4.7 % and this indicating that crossbreeding in rabbits was associated with a little improvement in the carcass performance. In Saudi Arabia, Al-Saef et al (2008) showed non-favorable negative estimates of maternal heterosis of -65.5 g, -6.7 g, -5.3 g and –12.2 g for hot carcass, offal, fat and bone weights, respectively; i.e. crossbred dams gave significant negative maternal heterotic effects on carcass traits ranging from 4.8 to 18.7%. For meat quality traits, neither individual heterosis, nor maternal heterosis were significant.

For semen parameters, direct heterosis given by Khalil et al (2007) indicated that crossbred bucks were associated with an existence of heterotic effects in some semen parameters. Such crossing was associated with an increase in ejaculate volume (11.6%; $P<0.05$), sperm concentration (10.5 %; $P<0.05$), percentages of motile (9.8%) and living sperms, and libido of bucks ($P<0.05$) along with a reduction in percentages of abnormal (-10.8%) and dead sperms (-23.5%; $P<0.05$). Reviewed estimates of maternal heterosis for semen characteristics were favourable and moderate (Khalil et al, 2007); indicating that crossbred dams gave maternal heterotic effects on some semen parameters in their progeny of crossbred bucks. Consequently, crossbred dams could produce crossbred bucks characterized by higher volume of ejaculate with more concentration and motile sperms, along with lesser percentages of abnormal sperms and dead sperms than their crossbred daughters.

Molecular analyses

Molecular analyses for economic traits to be used in genetic improvement program in rabbits are very limited. In this concept, RAPD technique is one of the most widely used techniques in applications of molecular biology for identifying the markers linked to traits of interest without the necessity for mapping the entire genome (Bardakci, 2001). RAPD marker of OPF09700 explained variation ranged from 10 to 14.7% for rabbit litter weight, gains, pre-weaning mortality and milk yield, while OPF12900 marker explained 14.7 and 16.8% of the variation for body weight at 4 and 8 weeks of age, respectively (Khalil et al., 2008). In practice, Microsatellite DNA is currently the most useful marker of choice for a wide range of molecular genetic studies in rabbits. Before building the genetic linkage map for QTLs for important traits in local and synthesized breeds, we must make some preliminary tests for variability within the breed and knowing which markers can be used and will be informative. The markers must be polymorphic to be useful. Actually, there were 305 initial sequences (AJ874368–AJ874672), a set of 183 were cytogenetically mapped in rabbits by Chantry-Darmon et al. (2006) and they are referred as INRA microsatellites. The second set were seven microsatellite sequences (D1Utr4, D3Utr2, D5Utr3, D6Utr4, D7Utr4, D12Utr2 and D19Utr2) and this assigned to chromosomes 1, 3, 5, 6, 7, 12 and 19, respectively (Korstanje et al. 2001, 2003). These markers are referred as Utrecht microsatellites. However, the first step of developing the genomic resources of rabbits was taken in August 2004 with the announcement by the National Human Genome Research Institute (USA) of a program for the partial sequencing of the rabbit genome, together with that of eight other mammals (http://www.nih.gov/news/pr/aug2004/nhgri-04.htm).

Till now, marker-assisted selection (MAS) is not generally used in current rabbits' selection programs in developing countries and the recent molecular technologies were used only in these countries to detect the associations between phenotypic traits and genetic markers. Khalil *et al.* (2008) used RAPD markers to search for the linkage between markers and quantitative traits and they used 526 rabbits in this analysis from a sire-granddaughters design in their selection program. From a total of 40 primers (10-mer) used in their study, five primers (OPA12, OPA19, OPA20, OPF09, and OPF12) were able to identify five polymorphic fragments at molecular weights of 1500, 1100, 1200, 700 and 900 bp, respectively, and only three markers of these markers ($OPF12_{900}$, $OPA19_{1100}$, and $OPF09_{700}$) showed significant associations with phenotypic traits, which indicated the presence of linkage between the three markers for litter and lactation traits and body weight at 4 and 8 weeks of age. Recently, El-Zarei (2010) detected five markers to differentiate between individuals for carcass traits and to study the association between these markers and some carcass, tissues composition traits and meat quality traits.

Genomic selection (Applications and Methodology)

Implementing genomic selection conceptually proceeds in the following steps:

1. Estimation of the effects of chromosome segments in a reference population.
2. Prediction of genomic EBVs (GEBVs) for animals not in the reference population, for example selection candidates.
3. Three distinct categories of animals (training, validation, and application) are involved in WGS. In practice whole genome selection has been applied successfully to the selection of AI young bulls in the dairy industry.

For analyzing SNPs data, Verbyla et al. (2010) reported that at each loci (total number of locus, p) there are three possible combinations of two alleles (e.g. A or B), the homozygote of one allele (AA), the heterozygote (AB) and the homozygote of the other allele (BB). These are then quantitatively represented by 0, 1 and 2 respectively. Subsequently, phenotypic records at each time point were modelled as:

$$y = \mu 1_{\mathrm{n}} + \sum_{j=1}^{q} X_j \beta_j + Zu = e$$

where y is the vector of phenotypes of the trait being analysed for all n individuals, μ is the mean, 1n is a vector of ones of length n, Xj is a vector of indicator variables representing the genotypes of the jth marker for all individuals (xij=0,1,2), βj is the size of the QTL effect associated with marker j, u is the vector of random polygenic effects of length n (Z is the associated design matrix) and is assumed to be normally distributed, $u \sim N\ (0,\ \delta^2 u\ A)$ where A is the pedigree derived additive genetic relationship matrix and e is the residual error also assumed to be normally distributed, $e \sim N(0, I\ \delta^2 e)$ where I is the nxn identity matrix. The prior distributions for the variances of the random polygenic effects and the residual were uninformative flat priors of the form $X^{-2}(-2, 0)$. The GEBV at each time point were calculated as:

$$\mathrm{GEBV} = \hat{\mu} + \mathbf{X}\hat{\beta} + \hat{u}.$$

Genomic selection (Advantages)

Whole genome selection (WGS) has the following advantages:

1) It is an approach to use DNA markers that are distributed throughout the entire genome. Genes affecting the most economic important traits are distributed throughout the genome and there are relatively few that have large effects with many more genes with progressively smaller effects.

2) Traditional marker-assisted selection (MAS) focuses only on those regions which are relatively certain to influence the trait of interest and leaves most of the genome and much of the genetic variation unaccounted for, while whole genome selection puts the greatest emphasis on those regions with the largest effects (that we can be most certain of) and still accounting appropriately for the more ambiguous genetic variation in the remainder of the genome.

3) It uses genotypes of thousands of single nucleotide polymorphism markers (SNP), like those from the 50,000 (50K) SNP chip, to predict breeding values (EBVs).

4) It is similar to the marker sets for DNA tests being offered now, but with much more density throughout the genome. Therefore, it allows SNPs with smaller effects on target traits to be used effectively. In theory, this will allow WGS to account for a greater proportion of genetic variation.
5) The same set of SNP could be used for all traits, because the SNP in the test span the entire genome.
6) Genomic selection is not a replacement for our current procedures and programs, but it will enhance our current programs by providing more reliable PTAs for young animals (especially sires). This will lead to more use of younger AI sires with moderately reliable PTAs and increase genetic progress for production traits as well as for health traits, reproductive traits and longevity.
7) Genomic selection will change breeding programs in rabbits and other small animals in the near future, already happens in dairy cattle since it has the same reliability for all traits in both sexes and it will shorten the generation intervals.
8) It improves the possibilities to select for functional traits (e.g. health, fertility) and improves also the selection against unfavorable recessive alleles.
9) Utilizing the genomic relationships and it provides new tools for risk management in breeding programs

Genomic selection (Disadvantages)

1) As compared to current DNA tests based on dozens to hundreds of SNP, whole genome selection would have greater cost per animal, because it uses the 50K chip.
2) Still need relatives for good EBV's and breeding programs.
3) Animals will be selected when sexually mature.

Updated future research work of genetic improvement programs

In order to produce a new synthetic multipurpose line in Egypt, we have to use three-way crossing among lines synthesized recently in Egypt (APRI, Moshtohor and Alexandria) and to use the genomic selection in such breeding program**.** To achieve this event, APRI bucks will be crossed with Moshtohor does to get the F_1 cross of ½APRI½M, then does of this F_1 cross will be crossed with Alexandria bucks to get ½A¼APRI¼M and then the progeny of this cross will be inter-se mated $(½A¼APRI¼M)^2$ for three generations to produce the multi-purpose line (Figure 1). Across generations, animals will be selected using a BLUP genome methodology.

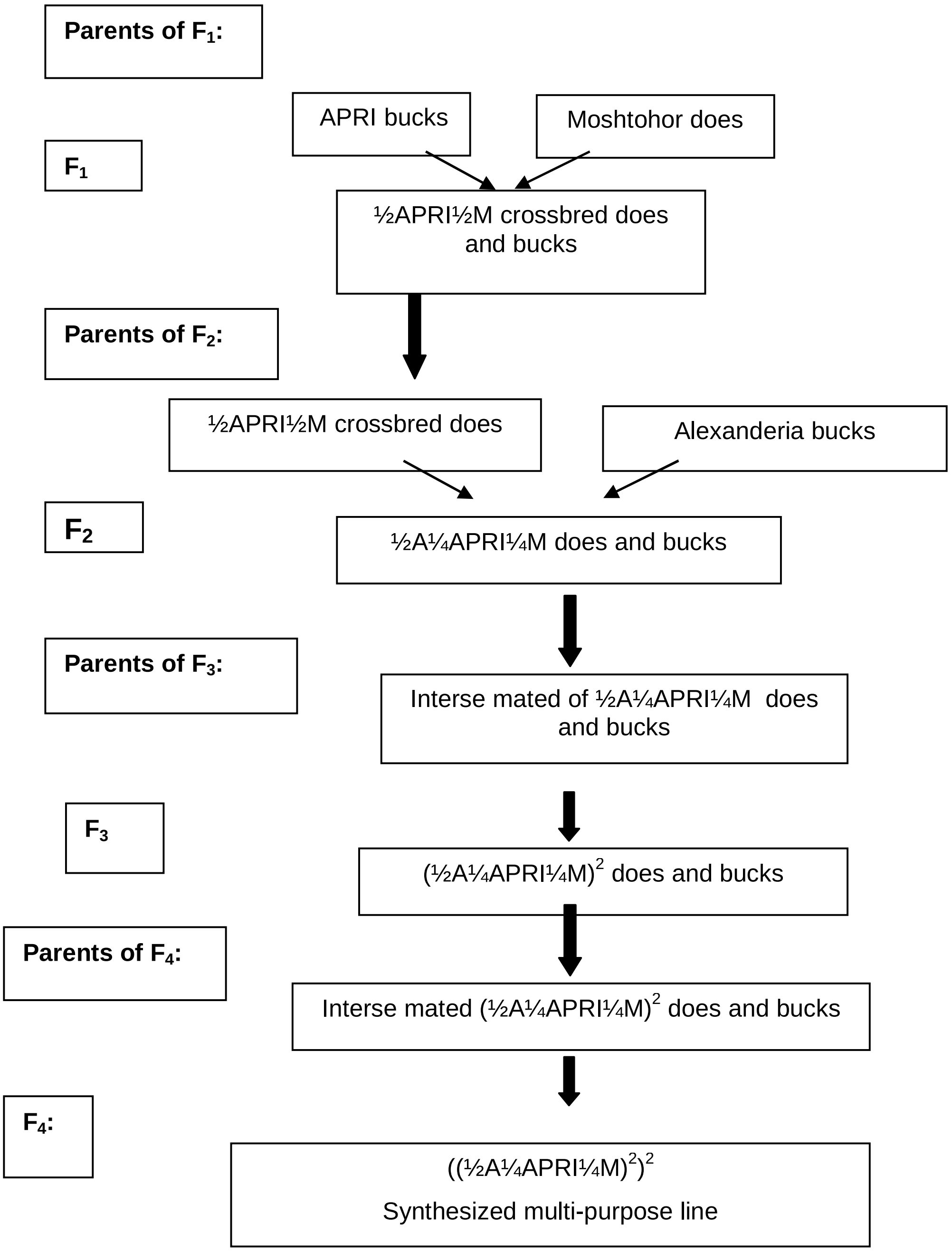

Figure 1: Diagram of three-way crossing to synthese the multi-purpose line.

CONCLUSIONS

1) Specialized maternal or paternal lines were recently developed to be used in Egypt on commercial scale.
2) Synthesizing multi-purpose lines are necessary to be established in the national rabbit industry.

3) The favourable estimates of direct and maternal heterosis reviewed for lactation, growth and carcass traits and heat-stress physiological parameters would be an encouraging factor for the rabbit producers in hot climate countries to use crossbred does and dams on commercial scale (Khalil et al, 2002; Al-Saef et al, 2008).
4) Insignificant recombination effects for milk traits and growth traits gave an impression to conclude that crossbred does resulting from crossing V-line with native breeds of rabbits in hot climate countries could be effective to develop multipurpose synthetic lines having more available heterosis to be used in commercial production in hot countries.
5) Till now, marker-assisted selection (MAS) is not generally used in current rabbits' selection programs in developing countries and the recent molecular technologies were used only in these countries to identify the associations between phenotypic traits and genetic markers (Khalil, 2008).
6) Genomic selection is much better than classical BLUP if there are no known relatives, and just better if there are (as reported by Legarra et al., 2008).

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نظرة إلى الطرز الجديدة المستنبطة من برامج التحسين الوراثي التي أجريت في مصر والمملكة العربية السعودية

ماهر حسب النبي خليل
كلية الزراعة بمشتهر – جامعة بنها
مشتهر ١٣٧٣٦ القليوبية