Evaluation of doe traits in a project of synthesizing new line of rabbits in Egypt

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Abstract

The experimental work of this study was carried out within a project of "improvement of rabbit lines" with the cooperation of the Spanish agency of international cooperation through four successive seasons from 2007 to 2008 in the rabbitry of the Department of Animal Production, Faculty of Agriculture, Benha University, Egypt. A total number of 45 does in three breed groups of Gabali, V-line and Moshtohor line (M-line) rabbits. Three does from each breed group were chosen randomly and sacrificed at 12 day of gestation (after palpation and pregnancy assurance). The studied were ovulation rates (OR), number of implantation sites/doe (NIS), number of viable embryos/doe (NVE), implantation rate (IR), number of corpora lutea (NCL), weight of corpora lutea (WCL), weight of lutein tissue (WLT), weight of ovary (WO), early embryonic mortality (EEM) % and some histological studies in uterine horn. Obtained results showed significant differences in OR, NIS, NVE, IR, NCL, WCL and WLT between the Gabali, V-line and M-line being 8.67, 8.1 and 9.2 for OR; 7.36, 7.43 and 8.33 for NIS; 5.02, 6.67 and 7.13 for NVE; 84.89%, 91.72% and 90.54% for IR; 12.9, 14.1 and 13.6 mg for WCL; and 111.84, 114.21 and 125.12 mg for WLT, respectively. Average of the early embryonic mortality was 20.69, 9.12 and 10.87% in Gabali, V-line and M-line, respectively. However no significant difference was found in weight of ovary due to the effect of breed group. The mucosal layer of uterus was lined by columnar epithelium with underlying connective tissue of the lamina propria which was rich by dilated blood capillaries in V-line and M-line than in Gabali breed. The muscular layer was thicker in M-line does than in both Gabali and V-line. It is concluded that doe rabbits of M-line had superiority in OR (9.7%), INS (12.6%), NVE (22.0%), IR (2.5%), NCL (9.7%), WCL (0.74%), WLT (10.7%), WO (-1.2%) and EEM (-27.1%) over of the two foundations.

Key Words: Doe and histological traits, rabbits, heterosis for doe traits, synthetic line of Moshtohor.

Introduction

One of the most interesting principles in the rabbit management is the selection for the suitable breeds or lines using in crossbreeding programs to maximize meat production. Selection on uterine capacity could be a more efficient way for improving litter size, although more research is needed to confirm this possibility. The Gabali local breed living in north Sinai are well adapted to the Egyptian conditions (Iraqi *et al.*, 2008). The Spanish V-line exhibits an outstanding maternal ability as related to behavior, fecundity and lactation (Estany *et al.*, 1989 and Garcia *et al.*, 2000). Moshtohor line (M-line) was founded in 2006 (Iraqi et al., 2010 and Youssef *et al.*, 2008) as a synthesizing line between the Egyptian Sinai Gabali (50%), (Afifi, 2002) and the V-line (50%), (Estany et al., 1989). The procedure of foundation began by mating V line does to Sinai Gabali bucks and it was followed by three generations of "inter-se" mating. Afterwards the line has been selected to increase litter weight at weaning and individual weight at 56 day. The main objective of the crossbred is to produce larger numbers of rapidly growing rabbits than those could be produced by pure breeding (El-Maghawry et al., 1999). There is a high variation in the reproductive performance of does in Egypt, which could be attributed to management decision in terms of post-partum mating, remating schedule... etc (Khalil, 1993). Argente *et al.* (2003) found that the high line showed higher ovarian weight (0.08 g, $p<0.05$) linked to a higher ovulation rate. Also the number of implanted embryos and live fetuses, fetal survival, and uterine weight and length were positively associated and explained most of the observed variation. Litter traits are usually regarded as the best estimate of number and weight of young produced by the doe since they constitute functions of all pre-weaning effects. Litter weight at weaning, as a composite trait, reflects the contribution of fertility, maternal behavior, milk production, pre-weaning growth and survival (Lukefahr *et al.*, 1990). Jonson and Rask-Andersen (1993) clarified that weights of right and lift ovary in NZW rabbits ranged from 178 to 489 mg at 6 days post-coitum. Gosalvez *et al.* (1987) found that California multifarious does showed higher value of ovarian weight than precipitous (371.5 vs. 290.4 mg) reaching maximal weight (385.0 mg) from the 28th to 30th days of gestation and decreasing after kindling until a minimum value on the first day post-partum (243.6 mg). They added that, ovaries weight in rabbit does increased from 328.9 to 333.0 mg when the number of litters increased from 0-7 to 8-14 litters. Also, they reported that, the weights of the left and right ovaries were approximately close to each other (326.4 vs. 332.5 mg). The highest ovarian weight may be attributed to the increase in levels of FSH

and LH hormones which enhance development and growth of ovarian follicles and increase the number of growth or mature ova (Rommers *et al.,* 2004).Uterine capacity is defined as the maximal number of fetuses that the dam is able to support at birth when ovulation rate is not a limiting factor (Christenson *et al.,* 1987). Moce *et al.* (2004) noticed that the uterine capacity begins to affect litter size before implantation in rabbits; embryonic survival depends mainly on embryo viability and oviduct and uterine environment; available uterine space is a imitating component of fetal survival, which is related to an adequate vascular supply for nutrient exchange from the maternal to fetal blood streams and an adequate surface area for development of the placenta. Selection on uterine capacity could be a more efficient way of improving litter size in rabbits than direct selection on litter size, although more research is needed to confirm this possibility (Blasco *et al.,* 1994). Argente et al. (2003) found differences in doe traits between the high- and low uterine capacity in rabbit's lines.

After implantation, there is a critical moment for fetal survival in rabbits between the day 8 and 17 of gestation, when the hemochorial placenta of rabbit has finished its development and the nutrition of the fetus begins to be controlled by the placenta (Adams and Chang, 1960). It has been reported that 52% of the fetal losses takes place between implantation and day 18 of gestation for unilateral ovariectomized does (Moce *et al.,* 2004) and 29% from implantation to birth for intact does (Argente *et al.,* 2003).

Ovulation rate could be measured by counting the number of corpus luteum at days 15 post-coitum (Hafez and Hafez, 2000). Hulot and Mariana (1985) recorded the ovulation rate 8 hours after mating to be 13 and 11 for Cal and NZW female rabbits, respectively. Abd El-Kafy (2000) reported that in primiporous NZW rabbits, number of corpus luteum was 7.0 at 48 hrs. post-coitum, but less than that reported by Ismail *et al.* (1992), which was 10.5 in Bouscat does rabbits. The present work was carried out to investigate the effect of breed group of Gabali, V-line and M-line (as new synthetic line, 50% Gabali and 50% V-line) on reproductive activity at the 12 day post-coitum.

Materials and methods

The experimental work of this study was carried out within a project of "improvement of rabbit lines "with the cooperation of the Spanish agency of international cooperation through four successive seasons from 2007 to 2008 in the rabbitry farm of the Department of Animal Production, Faculty of Agriculture, Benha University, Egypt. In the base generation animals of V-line a synthetic maternal line originated in 1982 at the Department of Animal Science of Universidad Politecnica de Valencia, Spain. Litter size at weaning was considered as the criterion for selection in this line. The Moshtohor line (M-line) as an Egyptian synthetic line coming from a first cross between the Egyptian Sinai Gabali (50%) and the V-line (50%), followed by three consecutive generations of "inter se" mating (Iraqi *et al.,* 2010). Three does from each breed group were chosen randomly and sacrificed at 12 day of gestation (after palpation and pregnancy assurance). The weight of ovaries and uterus was recorded. Number of corpora lutea on the right and left ovary alongside the number and weight of survival embryos were recorded. The uterine horns were dissected surgically and embryos were examined for heart pulsation. The swelling in the horn were cut open and chorionic vesicles if present were transferred into a petri dish containing normal saline, carefully opened and embryos were released. Viable embryos were those showing spontaneous or induced heart beatings or those without any detectable symptoms of disintegration, e.g. their tone, color, proportion of the head and limbs in relation to the body and crown rump length were the same as for their counterparts with functioning hearts Embryos otherwise were considered dead. Swelling with partial or complete disintegrated embryonic membranes revealed typical cases of embryonic mortality. Ovarian weights, numbers of corpora lutea, implantation and viable embryos were recorded. Autopsy samples were taken from uterus of rabbits in different groups and fixed in 10% normal saline for twenty four hours. Washing was done in tap water then serial dilutions of alcohol (ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slide microtome. The obtained tissue sections were collected on glass slides, deparffinized and stained by hematoxylin and eosin stains (Banchroft et al., 1996) for histological examination through the electric light microscope. Statistical analysis: Data were analyzed using SAS Software (SAS, 2004) according to the following model:

$$Y_{ij} = \mu + B_i + e_{ij}$$

Where:

Y_{ij} = the j^{th} observation on the doe rabbit; μ = general mean, common element to all observations; B_i = the fixed effect of the i^{th} breed group and e_{ij}= random error associated with the individual observation. All data measured as percentages were subjected to arcsine transformation to approximate normal distribution before being analyzed. Tests of significance for the differences between means were carried out according to Duncan (1955).

Results and discussions

Reproductive activity

Both the number and weight of corpora lutea were determined. The data of ovulation rate estimated on the basis of counts of corpora lutea appear in Table 1. Rates for the three respective breeds when CL counts were done 1 days post coitum were 8.67, 8.1, and 9.2 in Gabali, V-line and M-line, respectively. Difference between mean was statistically significant ($p<0.05$) as presented in Table 2. Average numbers of implantations sites/doe were 7.36, 7.43, and 8.33 in Gabali, V-line and M-line, respectively. Difference between means was statistically significant ($p<0.01$) as presented in Table 2. Data on number of viable embryos/doe followed the same pattern reported for number of implantation (Table 1). Average number of viable embryos/doe was 5.02, 6.67, and 7.13 in Gabali, V-line and M-line respectively, showing significant differences ($p<0.05$) as presented in Table 2, of interest is the percentages of corpora lutea counted at 12 days post coitum associated with the formation of implantation sites. These percentages (implantation rate) were 84.89%, 91.72% and 90.54% in Gabali, V-line and M-line, respectively (Table 1), showing significant differences ($p<0.05$). Therefore, in this period the placenta would require an adequate surface area for its development and an adequate vascular supply for nutrient exchange from the maternal to fetal blood streams. After implantation, there is a critical moment for fetal survival in rabbits between the day 8 and 17 of gestation, when the hemochorial placenta of rabbit has finished its development and the nutrition of the fetus begins to be controlled by the placenta (Adams and Chang, 1960). Moce *et al.* (2004) noticed that the uterine capacity begins to affect litter size before implantation in rabbits; embryonic survival depends mainly on embryo viability and oviduct and uterine environment; available uterine space is a imitating component of fetal survival, which is related to an adequate vascular supply for nutrient exchange from the maternal to fetal blood streams and an adequate surface area for development of the placenta. These results are agreement with the findings of Garner *et al*, (1987), El-Terbany (1993), Ford *et al*. (2002). Valet *et al.* (2002), Argent *et al*, (2003) and Merchan *et al.* (2006) who found that, breed have significant effect on ovulation rates, number of implantation sites, number of viable embryos and implantation rate. Morphological differences for rabbit after 12 days post coitum: The means numbers of corpora lutea /doe are presented in Table 1. Results obtained show that average number of corpora lutea mounted 8.67, 8.1 and 9.2 in Gabali, V-line and M-line, respectively which show significant differences ($p<0.05$) as presented in Table 2 within the three studied breed groups. The average weights of corpora lutea mounted 12.9, 14.1 and 13.6 mg in Gabali,V-line and M-line, respectively which show significant differences within the three studied breed groups. However, the average weight of lutein tissue/doe mounted 111.84, 114.21and 125.12 mg in Gabali, V-line and M-line, respectively (Table 1). The weight of ovary means was 380, 440 and 405 mg in Gabali, V-line and M-line, respectively which show no significant differences due to breed group effect. Average of the early embryonic mortality was 20.69, 9.12 and 10.87% in Gabali, V-line and M-line, respectively. These results are in agreement with many studies El-Fouly *et al.* (1977), Gorbner *et al.* (1987), Radwan and El-Sayed (1990) and Blasco *et al.* (1994) who found that, breed have significant effect on number of corpora lutea /doe and weights of corpora lutea. On the other hand, results obtained disagreed with those of Hafez (1964) who reported that the average weight weights of corpora lutea pair in multifarious NZW does at 6 days post-coitus ranged from 266 to 892 mg with an average of 479 mg. There was no significant difference between the weight of ovaries in NZW and Chinchilla rabbits breeds.

Table 1. Least-square means of morphological traits in different breed group of rabbits after 12 days post coitum.

Observation (Imposed at 12 day post coitum)	No. of Obs.	Breed group[+]			Heterosis (%)
		Gabali	V-line	M-line	
Ovulation rates [(1)](OR)	9	8.67^{ab}±0.50	8.1^{b}±0.50	9.2^{a}±0.50	9.7
Number of implantation sites/doe(NIS)	9	7.36^{b}±0.63	7.43^{b}±0.63	8.33^{a}±0.63	12.6
Number of viable embryos/doe(NVE)	9	5.02^{b}±0.41	6.67^{b}±0.41	7.13^{a}±0.41	22.0
Implantation rate (IR) %	9	84.89	91.72	90.54	2.5
Number of corpora lutea /doe(NCL)	9	8.67^{ab}±0.50	8.1^{b}±0.50	9.2^{a}±0.50	9.7
Weight of corpora lutea (WCL), mg	9	12.9^{b}±0.001	14.1^{a}±0.001	13.6^{a}±0.001	0.74
Weight of lutein tissue/doe (WLT), mg	9	111.84	114.21	125.12	10.7
Weight of ovary (WO), mg	9	380	440	405	-1.2
Early embryonic mortality (EEM) %	9	20.69	9.12	10.87	-27.1

[+] Means with different superscript in the same row are significantly different at ($p<0.05$).

[++] Heterosis percentages were computed as: {(average of M-line – (average of the Gabali and V-line)) / (average of the Gabali and V-line)} x 100. [(1)]. Assessed by counting number of corpora lutea / doe.

Table 2. F-ratios of least squares analysis for breed group affecting morphological tarits[+] in doe rabbits after 12 days post coitum.

S.O.V	d.f.	F-ratios					
		OR	NIS	EAT	NCL	WCL	WO
Breed group	2	4.00*	1.73**	0.67*	4.00*	1.00*	1.75ns
Error d.f.	6						
Error MS		0.778	1.22	0.67	0.778	0.00005	0.001

n.s = non-significant, * = p<0.05 and ** = p<0.01.
[+]OR = *Ovulation rates* (1), NIS =*Number of implantation sites/doe*, EAT= *Number of embryos atresia*, NCL =*Number of corpora lutea /doe*, WCL =*Weight of corpora lutea (mg)*, WO =*Weight of ovary (mg)*.

On the other hand, results in Table 1 showed also that doe rabbits of M-line had superiority in OR (9.7%), INS (12.6%), NVE (22.0%), IR (2.5%), NCL (9.7%), WCL (0.74%), WLT (10.7%), WO (-1.2%) and EEM (-27.1%) over the two foundations. This may be due to dominance and/or over dominance of genes action. These results are agreement with foundations of Argente *et al.* (2003) and Moce *et al.* (2004).

Histological studies:
The mucosal layer of uterus was lined by columnar epithelium with underlying connective tissue of the lamina propria which was rich by dilated blood capillaries in V-line and M-line than in Gabali breed. Maximum uterine proliferation Table 3 was observed in the endometrium of V-line Figure, 1 and followed by M-line Figures 2 and 5. The contrary picture was observed in Gabali Figures, 3 and 6. Concerning the thickness of myometrium, inner circular and outer longitudinal muscular layer was recorded with outer serosal layer. The muscular layer was thicker in M-line than in Gabali and V-line. This seems to be associated with the reproductive activity.

Table 3. Histological findings recorded in the uterus in different breed groups of rabbits at 12 day of gestation.

Breed group	Uterus lesions			
	Hyperplasia of the epithelial lining of the endometrium	Activation of glands in endometrium	Congestion of blood vessels	Hyperplasia of the myometrial layer
Gabali	++	+	+	++
V-line	+++	+++	+++	+++
M-line	+++	+++	+++	+++

+ = low, ++ = moderate and +++ = High.

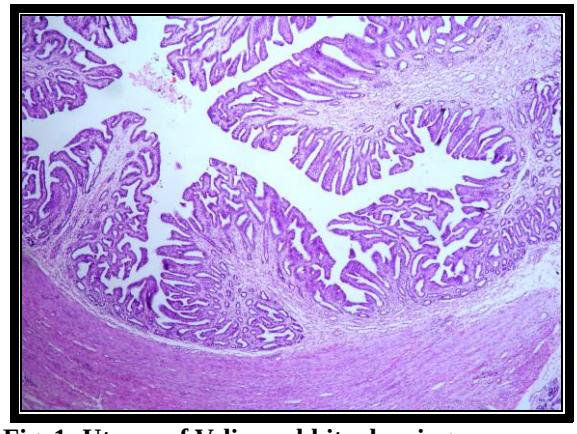

Fig. 1: Uterus of V-line rabbits showing hyperplasia of the epithelial Endometrium (H, E X40)

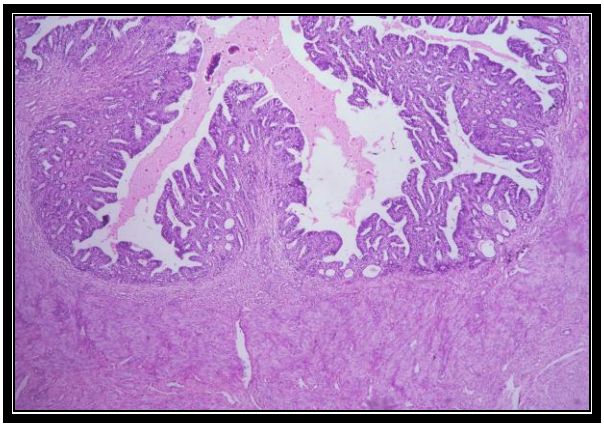

Fig. 2: Uterus of M-line rabbits showing hyperplasia of the epithelial Endometrium (H, E X40)

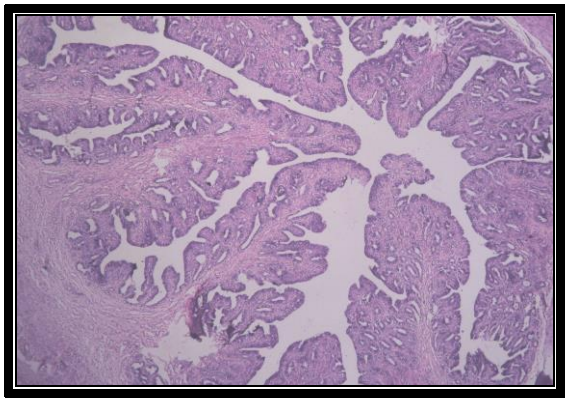

Fig. 3: Uterus of Gabali rabbits showing mild hyperplasia of the epithelial lining (H, E X40)

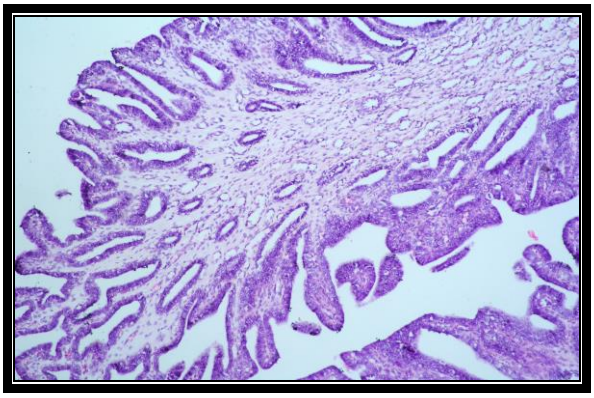

Fig . 4: Uterus of V-line rabbits showing activation of glands in endometrium. (H, E X40)

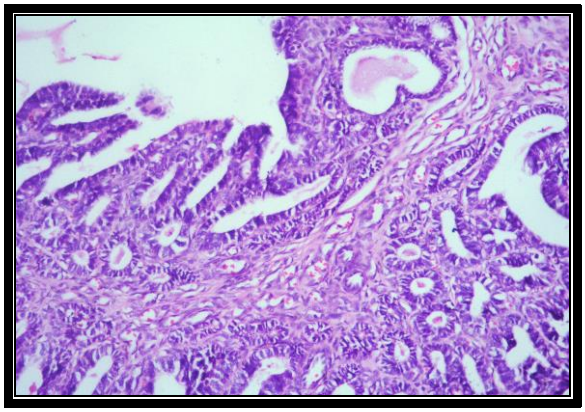

Fig. 5: Uterus of M-line rabbits showing activation gland and congestion of blood vessels. (H, E X40)

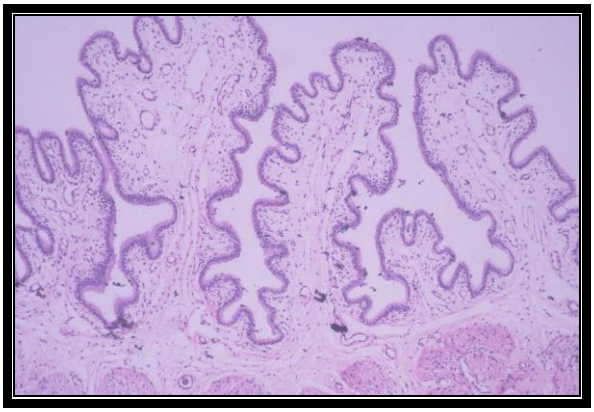

Fig. 6: Uterus of Gabali rabbits showing mild activation of glands. (H, EX40)

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