FEEDING REGIMEN DURING REARING IN FEMALE RABBITS UNDER EGYPTIAN ENVIRONMENTAL CONDITIONS: EFFECT ON OVARIAN ACTIVITY AND EMBRYO QUALITY PRODUCTION

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ABSTRACT

Forty New Zealand White rabbit does, ten weeks old with an average live body weight of 1605±10 g, were divided into four homogenous groups. The 1st group was fed *ad libitum* (control, C) and the 2nd group was fed *ad libitum* but fasted one day per week (F). The 3rd group was restricted fed (80% of *ad libitum*, R-1) while the last group was also restricted fed but they received their diet in two meals/day (at 8-9 am and 5-6 pm, R-2). All treatment groups were fed according to the treatment procedure until one week prior to the first insemination (22 weeks of age) to stimulate receptivity (flushing), afterwards they were all fed *ad libitum* the standard diet. At the end of the flushing period, does were intramuscularly injected with 20 µg GnRH and were inseminated with fresh semen. All does were slaughtered at 72-76 hr post AI. Daily weight gain was significantly ($P<0.001$) reduced in restricted and one day/week fasted groups. Compared to the control group, ovulation rate and number of CL on ovaries significantly ($P<0.05$) increased with restrictive diets. Fertilization rate was higher in R-2 group, followed by R-1 than in F and C groups, respectively, but differences were not significant. Relative weights of ovaries, oviducts and uterus were increased ($P<0.05$) under feeding regime. From the histological point of view, oviducts and uterus development were more pronounced with fed restriction groups, followed by F than C groups. Restricted fed does had an increased number of embryos recovered and significantly ($P<0.05$) higher percentages of embryo developed to early blastocyst stage with an excellent or good quality. It can be concluded that restricted regime given in two or one meal/day, with one week flushing period before the 1st insemination, enhances ovarian efficiency, genital organs development, and improves pre-implantation embryo development.

Key words: Rabbits, feeding regimen, ovarian efficiency, embryo survival.

INTRODUCTION

Nutrition may influence reproductive performance by number of mechanisms including central effects on gonadotropin secretion (Booth *et al*., 1994) and local effects on ovarian function (Cosgrove and Foxcroft, 1996; Meshreky *et al*., 2007). Determination of the nutritional needs in reproductive does has been intensively studied, but the direct influence of nutrition on the ovarian physiology is poorly known in rabbits. Some nutritional factors may influence oocyte maturation that is the first step affecting successful fertilization and preimplantational embryo development (Alvarez *et al*., 2008). Since there is a relationship between body reserves status of animals and their reproductive success, the effect of feeding strategy in young does until first insemination time should be tested. The effects of nutrition quantity on ovarian function have been reported in different species (Ferguson *et al*., 2006). However to our knowledge, there are few reports on the effects of nutrition on the ovarian function of rabbits. Therefore the aim of this study was to analyze the effects of different feeding strategies given out during the rearing period (from 10 weeks to first artificial insemination) on ovarian activity and embryo quality under Egyptian environmental conditions.

MATERIALS AND METHODS

Forty New Zealand White rabbit does, ten weeks old with an average live body weight of 1605±10 g, were divided into four homogenous groups. The 1st group was fed *ad libitum* (control, C) and the 2nd group was fed *ad libitum* but fasted one day per week (F). The 3rd group was restricted fed (80% of *ad libitum*, R-1) while the last group was also restricted fed but they received their diet in two meals/day (at 8-9 am and 5-6 pm, R-2). All groups except the control group, were fed according to the treatment procedure until one week prior to the first insemination (22 weeks of age) to stimulate receptivity (flushing), afterwards they were all fed *ad libitum* the standard diet. At the end of the flushing period, does were intramuscularly injected with 20 µg GnRh (Fertagyl) to induce ovulation and artificially inseminated (AI) with fresh semen. All does were slaughtered at 72-76 hr post AI. After the sacrifice of rabbits, genital organs (ovaries, oviducts and uterine horns) were weighed. Relative genital organs weight was calculated as follow: [(the weight of ovaries or oviducts or uterus (g) / live body weight (g) at 22 weeks of age)*100]. The oviducts and uterine horns were flushed in order to recover embryos and oocytes with DPBS with 0.132 g/l calcium chloride and 0.2% BSA at room temperature. Embryos were collected in a Petri dish, counted and evaluated under inverted microscope according to Lindner and Wright (1983) as follows (Figure 1): 1. Excellent- ideal embryo: spherical, symmetrical with cells of uniform size, colour and texture; 2. Good-trivial imperfections: a few extruded blastomeres with irregular shapes and few vesicles; 3. Fair-definite, but with no severe problems and 4. Poor-severe problems. Number of *corpora lutea* (CL) on ovaries was scored. Ovulation rate was calculated as percentage of females ovulating in each group. Fertilization rate was estimated as the ratio number of embryos recovered/total recovered (embryos + oocytes). For histological study, tissue samples from different parts of oviducts and uterus were taken and fixed in alcoholic Bouin's solution, processed by standard technique for paraffin wax embedment; sections are stained with haematexylin and eosin (H & E) as described by Bancroft and Cook (1994). The slides of oviducts and uterus were evaluated for development under light microscope. After recovery, embryos were evaluated according to their stage of development using an inverted microscope. At 72-76 hrs after AI, embryos were classified as compacted morulae and early blastocyst stages. Rabbits were raised in semi-closed rabbitry, fed a standard balanced pelleted diet according to NRC (1984) composed of 16.2% CP, 2.6% fat and 12.1% CF. Fresh water was offered *ad libitum* during all the day. The light regime used was about 16 hr/day (artificial light). Body weight and feed intake were recorded weekly. Data was statistically analyzed using the GLM Program of SAS (1998). Differences between means were analyzed by the Duncan's New Multiple Range (Duncan, 1955). Data presented in percentage was analyzed by chi square test.

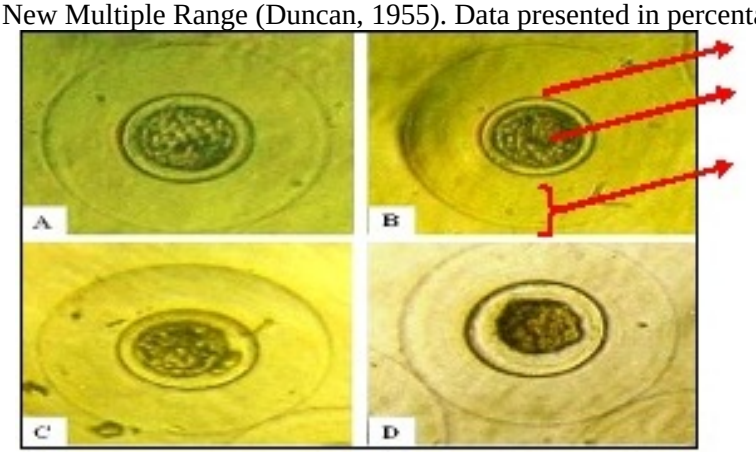

Figure 1: Developmental stages and quality of rabbit embryos recovered at 72-76 hrs post artificial insemination: (A & B) Excellent and good early blastocyst stage embryo, (C & D) Poor and fair compacted morulae stage embryo.

RESULTS AND DISCUSSION

Growth performance, genitalia organ weight and development

Daily weight gain was significantly (P<0.001) reduced in one day/week fasted and in both restricted groups (Table 1). Feed conversion was improved (P<0.01) by feed restriction particularly for R-2 group. These results are in agreement with those of Cervera *et al*. (2008) who found that feed restriction (12-18 weeks of age) affected (P<0.05) the live weight of young does at insemination (- 326 g of live weight *vs*. control group). Tumova *et al.* (2007) also observed that feed conversion is

improved by feed restriction. In our work, the body weight did not vary at the beginning of the experiment, but 12 weeks later the live body weight was higher (P<0.01) in does fed *ad libitum* during rearing, compared to others feeding programs. Conversely relative weights of ovaries, oviducts and uterus increased (P<0.05) under feeding regime, especially with restriction (Table 1). The increase in body weight of does fed *ad libitum* could be mainly caused by deposition of fat tissues. Restricted feeding can prevent excessive fat deposition in doe rabbits (Rommers, 2004). No mortality was observed, whatever the treatment during the experimental period. From the histological point of view, R-2 group showed normal appearance of fallopian tubes folds developments in the mucosa layer and more changes in number and maturation of uterine glands in the endometrium layer, followed by R-1 does which had more pronounced characteristics than those of F and C groups (Figure 2). The improvement in oviducts and uterine glands developments in R groups may be due to estradiol concentration (a biological active estrogen hormone produced by the ovary), wich acts on the uterus,

Table 1: Growth performance and relative genitalia organs weights of NZW doe rabbits as affected by feeding regimen

Feeding regimen	Body weight (kg) at		Daily body weight gain (g)	Daily feed intake (10-22 wk, g)	Feed conversion ratio (10-22 wk)	Relative weight†		
	10 wks	22 wks				ovaries	oviducts	uterus
Control	1.61	3.17^{a}	18.45^{a}	136.5^{a}	7.41^{a}	0.0144^{c}	0.035^{b}	0.175^{b}
Fasted	1.59	2.99^{b}	16.67^{b}	118.6^{b}	7.12^{b}	0.0154^{bc}	0.037^{b}	0.179^{b}
Restricted-1	1.61	2.86^{c}	14.83^{d}	105.4^{c}	7.13^{b}	0.0176^{a}	0.041^{a}	0.199^{a}
Restricted-2	1.61	2.95^{bc}	15.60^{c}	106.1^{c}	6.84^{c}	0.0166^{ab}	0.038^{ab}	0.187^{ab}
±S.E.	0.01	0.04	0.22	1.2	0.11	0.001	0.002	0.006
Significance	NS	**	***	***	**	*	*	*

† Relative weight was calculated as a percentage to body weight of rabbit does at 22 wks of age.
***: P<0.001, **: P<0.01, *: P<0.05 and NS = Not significant.
[a, b, c] Means with different superscripts in the same column, differed significantly (P<0.05).

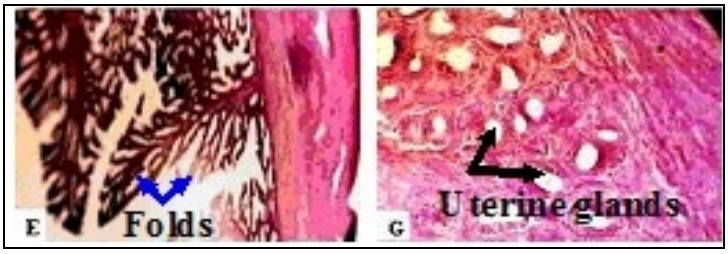

Figure 2: Cross sections stained with haematexylin and eosin in E) Fallopian tube of R-2 rabbits, showing normal appearance of the folds (x 33). G) Uterus of R-2 rabbits, showing more number and activity of the uterine glands in endometrium layer (x 132).

to increase the mass of both endometrium and myometrium (Hafez, 2000). Meshreky *et al*. (2012) found that 17ß-estradiol concentration significantly increased with restrictive diets of rabbit does during rearing. Rodriguez de Lara *et al*. (2000) also found that the increase in the availability and previous consumption of nutrients before the estrous flushing improves its appearance and the reproductive performance in does. However, Rebollar *et al*. (2011) observed that feed restriction (11–16 weeks) delays reproductive development in young rabbits inseminated at 17 wks of age. The differences between these studies could be due to differences in the level of feed restriction amount and/or duration or the earlier first insemination.

Ovarian activity, embryo developmental stages and quality

Compared to the control group, ovulation rate and number of CL on ovaries significantly (P<0.05) increased with restrictive diets (Table 2). No significant differences were observed between all groups in recovery rate. Fertilization rate was higher in R-2 group, followed by R-1 than in F and C groups respectively, but differences were not significant. The same trend was observed by Maertens (1995) who suggested that young does feed restriction until the 1st mating (18 weeks), with a short flushing period before insemination, significantly increases the number of follicles with a diameter exceeding 0.6 mm. The higher ovarian efficiency in the restrictive groups may be related to the better sexual

maturity achieved. Bonanno *et al.* (2004) found that sexual receptivity and pregnancy rate of NZW rabbits fed restricted diet and inseminated at 19.5 wks of age were higher than that fed *ad lib*. Rodriguez de Lara *et al.* (2000) suggested that in nulliparous does, flushing after a restricted diet, increases the amount of energy available, which could act on the hypothalamo-pituitary-ovarian axis.

Table 2: Ovarian activity, embryo developmental stages and quality (%), 72-76 hrs after the first insemination as affected by feeding regimen in NZW doe rabbits

Feeding regimen	Ovarian activity				Embryo developmental stages		Embryo quality	
	Ovulation rate (%)	Number of corpora lutea (CL)/does	Recovery rate no. (%)	Fertilization rate no. (%)	Compacted morulea no. (%)	Early blastocyst no. (%)	Excellent and good no. (%)	Fair and Poor no. (%)
Control	60^{b}	6.75^{c}±0.17	40 (100)	35 (87.5)	29 (82.9^{a})	6 (17.1^{b})	28 (80.0^{b})	7 (20.0^{a})
Fasted	70^{b}	7.00^{bc}±0.16	47 (95.9)	42 (89.4)	33 (78.6^{ab})	9 (21.4^{ab})	38 (90.5^{ab})	4 (9.5^{ab})
Restricted-1	90^{a}	7.20^{ab}±0.13	64 (98.5)	62 (96.9)	43 (69.4^{b})	19 (30.6^{a})	61 (98.4^{a})	1 (1.6^{b})
Restricted-2	90^{a}	7.60^{a}±0.13	66 (97.1)	65 (98.5)	42 (64.6^{b})	23 (35.4^{a})	62 (95.4^{a})	3 (4.6^{b})

$^{a, b, c}$ Means with different superscripts in the same column, differed significantly ($P<0.05$).

Restricted fed followed by a one week flushing before the first insemination, showed a positive effect on the number of embryos recovered and the percentage of embryos developed ($P<0.05$) to early blastocyst stage, with an excellent or good quality, compared to F and C groups respectively (Table 2). These results are in accordance with Meshreky *et al.* (2007) who found that restricted fed rabbits during rearing, delaying the first mating to 20 weeks, clearly improved embryo recovery and quality, compared to *ad libitum* fed rabbits, inseminated at 18 or 20 wks of age. Gomez *et al.* (2004) suggested that feeding level improved rabbit does reproduction performance in commercial farms. Nutrition may influence reproduction through oocyte maturation which implies both nuclear and cytoplasmic maturation (Yanagimachi, 1994), considered as an important step for normal embryo development after fertilization. Moreover, there is increasing evidence that the diet before mating can have an impact on embryo survival in different species (Ferguson *et al*., 2006).

CONCLUSION

Restricted regime (80% *ad libitum*) given in one or two mealtimes/day (at 8-9 am and 5-6 pm) during rearing period of young NZW rabbits (from 10 weeks of age) with a 1st insemination at 22 weeks, enhances ovarian efficiency and pre-implantation embryo development of the first reproductive cycle. These encouraging results should be confirmed on a larger scale over several reproductive cycles.

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