OVULATION INDUCTION IN DOE RABBITS AND CAFFEINE ADMINISTRATION DURING PREGNANCY TO INCREASE LITTER PERFORMANCE

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Abstract - The present study was conducted to investigate the effect of caffeine administration during pregnancy period on some reproductive and productive traits of hormonally traited New Zealand White (NZW) rabbits - Three groups of 20 does each (average body weight 3350±83 g) were injected with saline solution (control) or with either 40 IU PMSG plus 50 IU HCG (group 2) or 40 IU PMSG plus 20 µg GnRH (group 3). Each experimental group was divided into two sub-groups according to caffeine administration. The first sub-group was used without caffeine administration. The 2nd was administred with caffeine (500 mg/liter of drinking water) from day 1 until day 28 of pregnancy.

The hormonally treated does were significantly ($P< 0.01$ or 0.05) higher in total litter size at birth and daily weight gain from birth to 21 days than the control. However, insignificant differences were detected in conception rate, gestation length, litter weight, milk yield, litter weight gain, bunny weight, pre-weaning mortality rate and plasma T_3 and T_4 concentrations among the experimental groups.

Litter weight at birth and 21 days, milk yield at 3rd and 4 th week of lactation, daily weight gain from birth to 21 days of age and plasma T_4 concentration were increased significantly ($P<0.01$ or 0.05) by caffeine administration during pregnancy period. However, conception rate, litter size, litter weight gain, bunny weight, pre-wening mortality rate and plasma T_3 concentrations were not affected by caffeine administration.

INTRODUCTION

Ovulation in doe rabbits can be successfully induced by using synthetic gonadotrophin releasing hormones (ESPEY, 1982 ; FAN *et al.*, 1988 ; BONANNO *et al.*, 1990 ; CANALI *et al.*, 1990 ; ARMERO *et al.*, 1991 ; CASTELLINI *et al.*, 1991 ; BOURDILLON *et al.*, 1992). However, insufficient milk production for suckling kits obstacle its practical use.

The lactation curve of the domestic rabbit was shown to be affected by breed of doe (NIEHAUS and KOCAK, 1973 ; ORTIZ *et al.*, 1982 ; LUKEFAHR *et al.*, 1983), parity (ABO-ELEZZ *et al.*, 1981) and number of kits suckling (TORRES *et al.*, 1979 ; LUKEFAHR *et al.*, 1983). Kits from small litters are generally observed to grow faster than those from larger litters. This assumes that kits in smaller number receive more milk and result in faster growth rates. Recently WELSCH *et al.* (1988) and SHEFFIELD (1991) found that caffeine increased the hormonal response of mouse mammary tissue and subsequently improved milk yield.

The objective of this study was to investigate the effect of caffeine administration during pregnancy period on some productive and reproductive traits in hormonally treated New Zealand White rabbits.

MATERIAL AND METHODS

The experimental work was carried out in a Commercial Rabbitry, Meit Ghamr, Dakahliya Province. The laboratory work was conducted at Rabbits Research Unit, Institute of Efficient Productivity, Zagazig University, Egypt.

A number of 60 primiparous NZW doe rabbits (aged 5-6 months with a mean live weight 3350±83 g) were used in the present study. Does were divided randomly into three experimental groups with equal numbers (n=20). The first group was used as a control and each doe was injected with saline solution (1 ml of 0.09 % NaCl). The other two groups were injected intramuscularly with 40 IU PMSG (Fouigon, Intervet, Italia, S.R.L. Milano) followed 72 h later by 50 IU HCG (Corulon, Intervet Italia, S.R.L. Milano) or 20 µg GnRH (Gonadoreline, Fertagyl, Intervet Lab.) in the 2nd and 3rd groups, respectively. Each experimental group was divided into two sub-groups (n=10) according to caffein administration. The first sub-group was used without caffeine administration. The second was administered with caffeine (500 mg/litre of drinking water) from day 1 until day 28 of pregnancy. Water or water supplemented with caffeine were replaced daily. Caffeine was removed on day 28 of pregnancy (2 days before litters were due). Ten fertile bucks (6-7 months of age) were used for mating. Mating was carried out at random between does and bucks and each doe was transformed to the buck's cage to be mated and returned back to its cage after mating. The experimental rabbits were alloted in a windowed house. Flat desk cages were provided with nests for does and automatic drinker nipples and feeding throughs. All kindled kits were remained in the nests with their dams for suckling form birth up to weaning at 28 days. All rabbits were fed *ad iibitum* a commercial pelleted ration containing 17.50 % crude protein, 11.92 % crude fibre and 1.63 % fat. Clean tap water was available at all times. All animals were kept under the same mangerial, hygienic and environmental conditions.

Number of does copulated, number of pregnant does, conception rate (%), gestation length (days), litter size, litter weight (gm), bunny weight (gm) at birth, 21 and 28 days of age, litter weight gain, daily weight gain, pre-weaning mortality rate (%) form birth to 21 and 28 days of age and milk production at 1st, 2nd, 3rd and 4th week were studied. Daily milk intake was estimated by the method described by LUKEFAHR *et al.* (1983).

Blood samples were collected from marginal ear vein under vacuum in heparinized tubes (3 does from each experimental sub-group) after mating, 7, 18 and 28 days from mating. Blood samples were immediately centrifuged at 3000 r.p.m. for 10 minutes and plasma were separated, freezed under -20°C and kept until assyed for triiodothyronine (T_3) and tetraiodothyonine (T_4) concentrations by using radioimmunoassay technique (coated-tube kits, Diagnostic Products Corporation, Los Angeles, USA).

Data were statistically analysed by using factorial design according to SNEDECOR and COCHRAN (1982). Duncan's New Multiple Range Test (1955) was used for the multiple comparisons. Conception rate was analysed using the Contingency Tables according to EVERITT (1977). Pre-weaning mortality percentages were subjected to arc-sin transformation before being analysed in order to approximate normal scale distribution. Means were transformed to the original scale before being illustrated.

RESULTS AND DISCUSSION

Doe traits

Effect of hormonal treatments - Results in Table 1 show that the values of conception rate, litter size at 21 and 28 days, litter weight gain showed insignificant differences among the experimental groups (i.e. control, 40 IU PMSG plus 50 IU HCG and 40 IU PMSG plus 20 µg GnRH). However, total litter size at birth was significantly ($P<0.01$) higher in the hormonally treated groups than in the control. The present results agreed with those obtained by BONANNO *et al.* (1990), CANALI *et al.* (1990), CASTELLINI *et al.* (1991) and BOURDILLON *et al.*. (1992) that injection of rabbits with PMSG plus HCG or GnRH increased ovulation rate and fertility of NZW rabbits.

Effect of Caffeine administration - Does administered with caffeine showed the best values of milk yield. The differences were not significant at 1st and 2nd week of lactation. However, caffeine administration significantly improved ($P<0.05$) the milk yield at 3rd and 4th week of lactation (Table 1). TUCKER and REECE (1962) and SHEFFIELD (1991) suggested that the increased in lactational performance when caffeine was administered to the mice may be due to the increase in mammary developmental capacity during pregnancy. Morever, TUCKER (1981) also added that the total secretory capacity of the mammary gland was greater in caffeine fed mice.
Data in Table 1 also showed that litter weight at birth and day 21 of lactation was increased ($P< 0.05$) by caffeine administration during pregnancy period as compared to without caffeine administration. This increase was about 21.2%, 18.3% and 11.2% at birth, 21 and 28 days of lactation period, respectively. Because rabbits are born immature (compared to domestic animals such as cattle or pigs), they are completely dependent on their mothers for nutrition for approximately the first 2 weeks of life (until their eyes open at approximately 2 weeks of age). Thus, litter weight at this time would expected to be a useful index for lactational performance (KNIGHT *et al.*, 1986). The appearent increase in litter weight may be due to the increase of growth rate of offspring during lactation. SHEFFIELD (1991) indicated that caffeine treatment during pregnancy did not affect offspring directly, but it increased litter growth during subsequent lactation. Litter weight gain from birth up to 21 and 28 days was insignificantly heavier by caffeine administration.
The results of the present work also indicated that conception rate, gestation length and litter size were not affected by caffeine administration. The interaction effect between caffeine administration and hormonal treatments were not significant on all traits of doe rabbits (Table 1).

Offsprings traits

Effect of hormonal treatments - The mean bunny weight, daily weight gain and pre-weaning mortality rate are shown in Table 2. The values of mean bunny weight at birth, 21 and 28 days of age, daily weight gain from birth 28 days and pre-weaning mortality rate (stillbirth and from birth to 21 and 28 days of age) showed insignificant differences among the experimental groups. However, daily weight gain from birth to 21 days was significantly higher ($P< 0.05$) in the control group than in the hormonally treated groups. This may be attributed to the larger litter size obtained by hormonally treated groups. AFIFI *et al.* (1973) and EL-KELAWY (1993) postulated that the mean bunny weight at birth or at weaning decreased with increased of litter size at birth.

Effect of caffeine administration - Data in Table 2 showed that bunny weight, daily weight gain from birth up to 28 days and pre-wearing mortality rate were not affected by caffeine administration during pregnancy. However, daily weight gain from birth up to 21 days of age was significantly higher ($P<0.05$) in the untreated group than in the treated group with caffeine. The interactions between caffeine administration and superovulation treatments were significant ($P<0.05$) on daily weight gain from birth up to 21 and 28 days of age. This may be due to the increase in milk yield at 21 and 28 days of lactation, which produced more gain weight (Table 1).

Thyroid function

Effect of hormonal treatments - Plasma thyroxine (T_4) and triiodothyroxine (T_3) fluctuated between 41.87 to 85.14 and 1.63 to 2.62 ng/100 ml, respectively, throughout the pregnancy period. The mean level of T_4 was slightly higher at day 18 of pregnancy than at day of mating, 7 and 28 days after mating. The increase in T_4 level in rabbits may be due to its role in tissue synthesis, especially, in the pregnancy period and development of fetuses. Injection of doe rabbits with 40 IU PMSG plus 20 µg GnRH showed, in general, higher T_3 and T_4 concentrations than in the other groups (Figure 1).
However, the differences were not significant at all ages studied during pregnancy.

Figure 1 : Thyroxine (T_4) and triiodothyronine (T_3) in hormonally treated rabbits administrated with caffeine during pregnancy

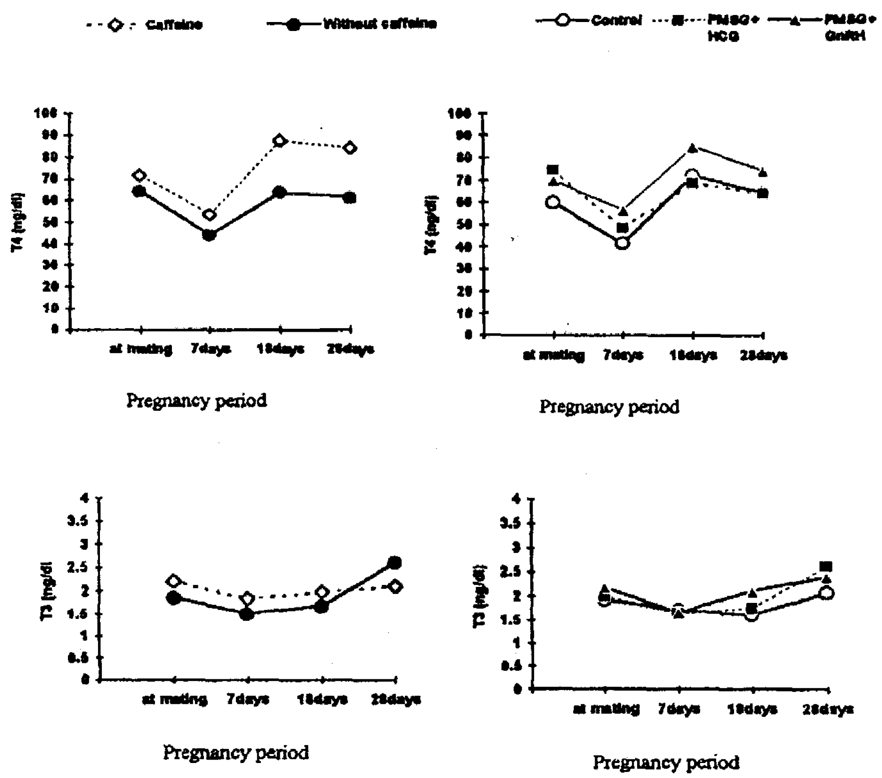

Table 1. Doe traits (x ± S.E) of hormonally treated doe rabbits administered with caffeine during preg

Treatments	No. of copulated does	No. of pregnant does	Conception rate (%)	Gestation length (days)	Litter size at				Milk yield (g) at		
					Birth		21 days	28 days	1st week	2nd week	3rd week
					Total	Live					
Hormonal treatments :			NS	NS	**	NS	NS	NS	NS	NS	NS
Control (A)	23	16	69.5	30.1±0.31	7.1±0.9 [b]	6.4±0.8	5.8±0.7	5.4±0.7	729.2±60.9	1137.5±69.9	1219.2±40.
40 IU PMSG+50 IU HCG (B)	19	15	78.9	30.7±0.16	11.0±0.5 [a]	9.3±0.6	7.0±0.5	6.2±0.5	770.0±75.6	1160.8±70.8	1335.8±66.
40 IU PMSG+20 µg GnRH (C)	18	14	77.8	30.4±0.40	10.1±0.9 [a]	8.5±0.7	6.4±0.4	6.2±0.4	758.3±63.7	1178.3±75.4	1400.0±51.
Caffeine administration:			NS	NS	NS	NS	NS	NS	NS	NS	*
Without caffeine (a)	32	23	71.9	30.4±0.26	9.4±0.9	8.3±0.8	6.6±0.6	6.0±0.5	727.2±51.8	1127.9±63.6	1252.2±46. [b]
With caffeine (b)	28	22	82.1	30.3±0.24	9.3±0.6	7.9±0.5	6.2±0.3	6.0±0.3	777.8±53.4	1190.0±47.8	1384.4±42. [a]
Interactions:			NS	NS	NS	NS	NS	NS	NS	NS	NS
A x a	13	9	69.2	30.0±0.53	6.3±0.2	5.6±1.3	5.5±1.5	5.0±1.3	700.0±122.9	1096.7±141.9	1143.3 ± 46.
A x b	10	7	70.0	30.1±0.26	8.0±1.3	7.1±0.9	6.0±0.7	5.7±0.5	758.3±50.9	1178.3±50.9	1295.0 ± 20.
B x a	10	7	70.0	30.4±0.30	11.9±0.8	10.3±0.7	7.7±0.9	6.6±0.8	746.7±121.8	1120.0±101.0	1260.0 ±106.
B x b	9	8	88.9	30.9±0.13	10.3±0.5	8.0±0.5	6.3±0.4	5.9±0.6	793.3±114.9	1201.7±114.9	1411.7 ± 70.
C x a	9	7	77.8	30.9±0.40	10.9±1.6	8.4±1.2	6.4±0.5	6.1±0.6	735.0±40.4	1166.7±129.9	1353.3 ± 30.
C x b	9	7	77.8	29.9±0.67	9.4±1.2	8.5±0.9	6.3±0.6	6.3±0.7	781.7±134.6	1190.0±106.9	1446.7 ±101.

Means in the same column in each classification having different letters, differ significantly (P< 0.05).
NS = Not significant * P< 0.05 ** P< 0.01

Table 2. Offspring traits (x ± S.E)of hormonally treated doe rabbits admin

Treatments	Offspring traits				
	Bunny weight (g) at			Daily weight gain (g) from	
	Birth	21 days	28 days	21 days	28 days
Hormonal treatments :	NS	NS	NS	*	NS
Control (A)	55.2±1.6	275±10.7	412±22.8	10.42±0.5 ^{a}	12.72±0
40 IU PMSG+50 IU HCG (B)	47.6±2.4	233±14.3	376±15.9	8.86±0.6 ^{b}	11.77±0
40 IU PMSG+20 μg GnRH (C)	53.6±3.7	240± 5.1	377±13.2	8.89±0.2 ^{b}	11.54±0
Caffeine administration:	NS	NS	NS	*	NS
Without caffeine (a)	47.9±2.4	234±9.4	377±16.0	8.86±0.4 ^{b}	11.77±0
With caffeine (b)	56.4±1.4	264±8.9	399±12.9	9.90±0.4 ^{a}	12.24±0
Interactions:	NS	NS	NS	*	*
A x a	51.6±2.1	276±17.1	499±32.3	10.64±0.7 ^{a}	14.16±1 ^{a}
A x b	59.2±0.9	274±14.8	381±28.8	10.23±0.7 ^{ab}	11.49±1 ^{bc}
B x a	39.6±1.7	196±8.3	330±15.1	7.44±0.4 ^{c}	10.39±0 ^{c}
B x b	54.5±2.1	270±18.9	422±12.9	10.29±0.9 ^{ab}	13.15±0 ^{ab}
C x a	51.8±6.2	236±5.3	363±14.7	8.75±0.3 ^{bc}	11.10±0 ^{bc}
C x b	55.6±3.9	246±9.2	393±22.5	9.06±0.4 ^{abc}	12.05±0 ^{ac}

Means in the same column in each classification having different letters, differ signi
NS = Not significant * $P< 0.05$

Effect of caffeine - Does administered with caffeine showed, in general, higher (P<0.05) T_4 and T_3 concentrations than in the control group. Peripheral plasma T_4 and T_3 levels in pregnant rabbits decreased steadily at day 7 of pregnancy, peaked at day 18 and declined on day 28 of pregnancy (Figure 1). The increase in T_4 and T_3 during pregnancy period indicates its vital role in energy metabolism to face milk synthesis (HABEEB and EL-MASRY, 1991). Thyroxine index T_4/T_3 was generally higher in the does administered with caffeine than in control during pregnancy, especially in the 2nd half of pregnancy. This indicate that T_4 and T_3 are important during pregnancy period. It can be concluded that rabbits try to regulate the hormonal pattern according to physiological status demands.

In conclusion, caffeine administration during pregnancy improved milk production in hormonally treated New Zealand White rabbits.

REFERENCES

ABO-ELEZZ Z.R., HASSAN A., SAMAK M., 1981. Effect of litter size and mating cycles on lactation in rabbits. *Alexandria J. Agric. Res.*, **29**, 75-82.

AFIFI E.A., EL-TAWIL E.A., GALAL E.S.E., EL-KHISHIN S.S., 1973. Some aspects of production in three breeds of rabbits and their crosses. I. Average individual weight per litter at birth. Annuals of Agriculture, Sciences, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, **18**, 32-41.

ARMERO E., PERIS FAJARNES G., GARCIA X.F., VICENTE J.S., 1991. Respuesta ovulation a la HCG en presencia no de cuerpos luteos previos en conejas de eliminacion. *Intormacion Tecnica Economica Agraria, Prd. Animal*, **87A**, 17-22.

BONANNO A., BUDETTA G., ALABISO M., ALICATA M., 1990. Effect of PMSG and GnRH treatment on the ovulatory efficiency of rabbits. *Actat Med. Vet.*, **36**, 441-451.

BOURDILLON A., CHMITELIN F., JARRIN D., PAREZ V., ROUILLERE H., 1992. Effect of PMSG treatment on breeding results of artificially inseminated rabbits. *J. Appl. Rabbit Res.*, **15**, 530-537.

CANALI C., BOITI C., CASTELLINI C., BATTAGLINI M., ZAMPINI D., 1990. PMSG and reproductive performance in rabbits. *Rivista di Coniglicoltura*, **27**, 21-23.

CASTELLINI J.C., CANALI C., BOITI C., BATTAGLINI M., 1991. Effect del PMSG sulle prestazioni ripoduttive di coniglie fecondate artificialmente. *Atti IX Congresso Nazionale ASPA*, pp. 679-683.

DUNCAN D.B., 1955. Multiple range and multiple F test. *Biometrics*, **11**, 1-42.

El-KELAWY H.M., 1993. Studies on reproductive and productive performance in rabbits. Ph.D. Thesis, Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

ESPEY L.L., 1982. Optimum time for administration of indomethacin to inhibit ovulation in the rabbits. *Prostagtandins*, **23**, 329-334.

EVERITT B.S., 1977. The Analysis of Contingency Tables. Monographs of Applied Propability and Statistics. pp. 38-66, London, Chopman Hall.

FAN B., XIONG H., SHAO C., 1988. Superovulation and embryo transfer in rabbits. *4th World Rabbit Congress, Budapest, Hungary.*

HABEEB A.A., EL-MASRY K.A., 1991. Hormonal pattern in pregnant rabbits and some productive aspects as affected by litter size at birth. Egypt. *Poult. Sci.*, **11**, 429-443.

KNIGHT C.H., MAITZ E., DOCHERTY A.H., 1986. Milk yield and composition in mice: effects of litter size and lactation number. *Comp. Biochem. Physiol.*, **84**, 127.

LUKEFAHR S., HOHENBOKEN W.D., CHEEKE P.R., PATTON N.M., 1983. Characterization of straightbred and crossbred rabbits for milk production and associative traits. J. Anim. Sci., 57, 1100-1110.

NEIHAUS V.H., KOCAK C., 1973. Milcheistungsprufugenbei Kalifornier - Hasinnen. *Arch. für Geflugeikunde*, **37**, 102.

ORTIZ R.B.R., TEWOIDE A.M., RODRIGUEZ C.A.A., 1982. Caraterizacion de la production de leche en conejos (Oryctolagus cuniculus L.) de las razas Nueva Zelanda y California. *Proc. 14th Reunion Anual de la Asociacion Mexicana de Produccion Animal, Universidad Autonoma Chapingo.* p.61 (Abstr.).

SHEFFIELD L.G., 1991. Caffeine administered during pregnancy augments subsequent lactation in mice. *J. Anim. Sci.*, **69**, 1128-1132.

SNEDECOR G.W., COCHRAN W.C., 1982. Statistical Methods. 8th Edition, Iowa State University Press, Ames, Iowa, U.S.A.

TORRES A., FRAGA M., DE BLAS J.C., 1979. Production de leche y mortalidad de los gazapos en la raza Neozelandesa. *Anales del Instituto Nacional de Investigaciones Agrarias, Production Animal*, **10**, 25.

TUCKER H.A., 1981. Physiological control of mammary growth, lactogenesis and lactation. *J. Dairy Sci.*, **64**, 1403.

TUCKER H.A., REECE R.P., 1962. Nucleic acid estimates of mammary tissue and nuclei. *Proc. Sco. Exp. Biol. Med.*, **111**, 639.

WEISCH C.W., DEHOOG J.V., O'CONNOR D.H., 1988. Influence of caffeine consumption on carcionmatous and normal mammary gland development in mice. *Cancer Res.*, **48**, 2078.