Hormonal and metabolic profiles of rabbit does subjected to fasting or calorie restriction

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Abstract: Forty New Zealand White rabbit does were used in this study. Rabbit does were assigned randomly to one of the following dietary treatments: (1) control group which received feed *ad libitum*, (2) restricted group which received 60% of total amount of feed consumed by control group (treatment lasted for four weeks), (3) restricted group which received 60% of total amount of feed consumed by control group (treatment lasted for eight weeks), (4) fasting for 24 hour group, and (5) fasting for 48 hour group. Hormonal profiles included estradiol 17β, progesterone, and Insulin-like growth factor one (IGF-1) were assessed. Significant reduction ($P<.01$) in estradiol 17β (E_2) was observed in all experimental groups compared to *ad lib* group. Fasting for 48 hour group showed the lowest plasma level of E_2 compared with other groups. There were no significant differences among experimental groups with regard to plasma levels of progesterone. However, feeding regimens did not affect IGF-1 plasma levels in all rabbit groups. Concerning biochemical profile, plasma levels of glucose and total proteins were not statistically affected by fasting or calorie restriction. Significant reductions ($P<.01$) in plasma triglycerides were observed in does subjected to feed deprivation for 24 or 48 hours compared to *ad lib.* and calorie restriction groups. On the other hand, no significant difference in plasma level of triglycerides was noticed between *ad lib.* and calorie restriction does. The results suggested that fasting as an acute nutritional insult induced more drastic effect on reproductive hormones than prolonged calorie restriction.

Keywords: New Zealand White, Hormonal profiles, estradiol 17β, progesterone, Insulin-like growth factor

INTRODUCTION

Several studies have pointed out to the role of nutrition on mammalian reproductive functions. Limited feed resources can affect reproductive efficiency in many animal species (Diskin *et al.*, 2003). Low, moderate, and high levels of dietary energy intake can affect reproductive function in different ways (Mackey, *et al.*, 2000). Severe calorie restriction was associated with failure of ovulation as a result of absence of pro-ovulatory LH and FSH surges. Undernutrition can suppress the hypothalamic - pituitary gonadal axis through its central inhibition (Ottinger *et al.*, 2005).

Recent studies indicate that hormonal and biochemical metabolic mediators including insulin, growth hormone, Insulin-like growth factor one IGF1, Insulin-like growth factor binding protein IGFBP, and glucose can modulate the effect of undernutrition on reproductive function (Rommers *et al.*, 2004).

The aim of the current study was to assess the reproductive hormonal profile as well as some biochemical responses of does subjected to fasting or calorie restriction.

MATERIALS AND METHODS

Animals: Forty adult female New Zealand White rabbits weighing 2.7-3 kg were used in this study. Animals were housed in individual cages in metal batteries. Female rabbits were provided feed and water *ad libitum*. Rabbits were maintained under 16 h light and 8 hr dark throughout the study.

Calorie restriction treatments: Rabbit does were assigned randomly to one of the following dietary treatments: (1) control group which received feed *ad libitum*, (2) restricted group which received 60% of total amount of feed consumed by control group (treatment lasted for four weeks), (3) restricted group which received 60% of total amount of feed consumed by control group (treatment lasted for eight weeks), (4) fasting for 24 hour group, and (5) fasting for 48 hour group. Rabbit does were fed basic diet contained 18% crude protein, 2.69% ether extract, and 13% crude fiber.

Blood samples: Does were sampled at the end of the restriction period and at the end of fasting period. Blood samples were collected from ear vein into heparinized tubes. Plasma samples were separated by centrifugation of blood at 3000 rpm for 10 min. and stored at -20°C for later hormonal and biochemical analysis.

Hormonal determination: Circulating levels of estrogen and progesterone hormones were analyzed by radioimmunoassay. Measurements determined using COAT A COUNT kits purchased from Diagnostic Product Corporation, U.S.A. Plasma level of Insulin-like growth factor one (IGF-1) was measured using ELISA kit purchased from DIAsource ImmunoAssays S.A. Belgum.

Biochemical determination: Plasma biochemical parameters were determined using commercially available kits which rely on colorimetric procedures. Plasma values of total protein, triglycerides, and glucose were measured using Stanbio kits (USA).

Statistical analysis: Data were statistically analyzed using one way analysis of variance (ANOVA) according to Steel and Torrie, (1980). ANOVA was performed using general linear model option available in SAS software (SAS, 1986). Differences among treatment means were tested using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

The effect of feeding regimens on plasma hormonal levels is summarized in Table (1). Feed deprivation for 24 hours resulted in a significant decrease in plasma estrogen levels. About 50% reduction in estrogen level was observed in one day fasting does compared to *ad lib*

control. Acute effect on plasma estrogen was recorded after imposing 48 hours fasting in rabbit does. Almost 60% reduction in estrogen level was associated with 48 hours deprivation of feed compared to *ad lib* does. It has been observed that even short term fasting can inhibit gonadal axis (Bonanno *et al.,* 2002). One day fasting resulted in lowering LH peak and reduced estradiol-17β pulse frequency and amplitude (Brecchia *et al.,* 2004). Similar results have been recorded for some species including primates and rats (Schreihofer *et al.,* 1993 ; Maeda *et al.,* 1994).

60% of *ad lib* calorie restriction either for one or two months showed significant reduction in circulated estradiol 17β. Longer duration of calorie restriction exerts more reduction in estradiol 17β compared to shorter duration.

Although fasting and calorie restriction had a similar trend toward lowering E_2 level, fasting had more drastic effect on estradiol level.

With regard to plasma progesterone (P_4) level, neither fasting nor calorie restriction exerted any significant effect on its level. Similar results were reported by Chiericato et. al 2001 where plasma P_4 value did not change by feed restriction in rabbit does.

Nutrition may influence reproduction performance by number of mechanisms, including central effects on gonadotropin secretion (Mao *et al.,* 1999). It has been suggested that metabolic mediators including insulin, growth hormone, leptin, IGF-1, and IGFBP may modulate the effects of nutrition on hypothalamic-pituitary ovarian axis (Funston *et al.,* 1995).

In the present study, circulating levels of IGF-1 were not affected by feeding regimens. In literature there are some contradicted results concerning plasma levels of IGF-1 and nutritional status. Straus *et al.,* 1993 and Jousse *et al.,* 1998 reported significant decrease in plasma IGF-1 in protein deficiency diet. On the other hand, Armstrong *et al.,* 2001 found that plasma levels were unaffected by protein concentration in the diet. Although circulating levels of IGF-1in the present study were not significantly affected by dietary regimens, there was a trend towards lowering IGF-1in does subjected to fasting. The current observed reduction in estradiol levels could be explained as IGF-1 might interfere with follicular development and steroidogensis. IGF-1 might have direct effect on ovary rather than central nervous system as indicated by presence of its receptors on ovarian membranes (Yoshimura *et al.,* 1996).

With regard to the biochemical profile, plasma levels of glucose and total protein were not affected by fasting or calorie restriction. These results are in agreement with what was found by Brecchia *et al.,* 2006. Although some findings indicated that glucose might serve as a specific mediator for the effects of calorie intake on reproduction, the mechanism by which glucose affect reproduction is not fully understood. Plasma levels of triglycerides were significantly decreased in fasted does compared with calorie restriction and *ad lib.* feeding groups. There were no significant differences between calorie restriction and *ad lib.* feeding groups. Brecchia *et al.,* 2006 reported significant elevation of non esterified fatty acids in rabbit does subjected to fasting. Decreasing levels of triglycerides in fasting does indicates shifting in metabolism from carbohydrate to fat in order to restore normal levels of glucose during fasting.

Although recent findings have provided considerable insight into the actions of undernutrition on reproductive system, the mechanism by which undernutrition exerts its effects remain unclear. Further studies are needed to disclose its action.

Table (1): Effect of feeding regimens on plasma estradiol 17β (E_2), progesterone (P_4), and insulin like growth factor one (IGF-1) in rabbit does (Means±SE).

Feeding Regimens	E_2 (pg/ml)	P_4 (ng/ml)	IGF-1 (pg/ml)
Fasting for 24 hours	43.813±2.721^{d}	0.391±0.023	351.527±19.381
Fasting for 48 hours	29.959±1.959^{e}	0.386±0.053	338.823±11.935
60% of *ad lib.* feeding for one month	67.379±3.985^{b}	0.341±0.048	445.223±51.787
60% of *ad lib.* feeding for two months	55.692±2.548^{c}	0.395±0.097	392.817±41.522
***ad lib.* feeding**	85.498±3.323^{a}	0.389±0.038	429.067±36.952

Means within a column with no common superscript are significantly different (P<.01)

Table (2): Effect of feeding regimens on plasma glucose, triglyceride, and total protein in rabbit does (Means±SE).

Feeding Regimens	Glucose (mg/dl)	Triglyceride (mg/dl)	Total protein (g/dl)
Fasting for 24 hours	112.50±2.89	084.53±6.21^{b}	6.79±0.44
Fasting for 48 hours	115.02±2.91	079.28±3.56^{b}	7.04±0.21
60% of *ad lib.* feeding for one month	116.81±6.37	108.98±4.73^{a}	6.29±0.35
60% of *ad lib.* feeding for two months	111.46±4.34	099.53±5.41^{a}	6.67±0.19
***ad lib.* feeding**	115.01±2.89	110.01±6.65^{a}	6.93±0.31

Means within a column with no common superscript are significantly different (P<.01)

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الصورة الهرمونية والايضية لإناث الأرانب الخاضعة للتصويم أو لتحديد الغذاء

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أربعون أنثي من الأرانب النيوزيلاندي الأبيض قد تم استخدامها في هذه الدراسة. تم توزيع هذه الأرانب عشوائيا علي المعاملات الغذائية التالية: المجوعة الأولي وقد تم تغذيتها علي العلائق بصورة حرة (المجموعة الضابطة) والمجموعة الثانية وقد تم تغذيتها علي ٦٠% من الغذاء المأكول بواسطة المجموعة الضابطة لمدة شهر والمجموعة الثالثة قد تم تغذيتها علي ٦٠% من الغذاء المأكول بواسطة المجموعة الضابطة لمدة شهرين والمجموعة الرابعة قد تم تصويمها لمدة ٢٤ ساعة والمجموعة الخامسة قد تم تصويمها لمدة ٤٨ ساعة. تم قياس هرمونات الأيستروجين والبروجيسترون وعامل النمو المشابه للأنسولين. لوحظ انخفاض معنوي في مستوي هرمون الايستروجين في كل مجموعات المعاملات التجريبية بالمقارنة بالمجموعة الضابطة. أظهرت مجموعة التصويم لمدة ٤٨ ساعة أكبر انخفاض في مستوي هرمون الايستروجين مقارنة بباقي المجموعات الاخري. لم توجد أي اختلافات معنوية بين المجموعات التجريبية بالنسبة لهرمون البروجيستيرون وهرمون عامل النمو المشابه للأنسولين.

بالنسبة للقياسات البيوكيميائية لم تتأثر مستويات بلازما الجلوكوز والبروتين الكلي معنويا بالتصويم أو بتحديد الغذاء. لوحظ انخفاض معنوي في مستويات بلازما الجليسريدات الثلاثية في مجموعتي التصويم لمدة ٢٤ أو ٤٨ ساعة بالمقارنة بالمجموعة الضابطة ومجموعة تحديد الغذاء. لم يكن هناك فروق معنوية بين المجموعة الضابطة ومجموعة تحديد الغذاء بالنسبة لمستويات الجليسريدات الثلاثية. النتائج الحالية تقترح أن التصويم يؤدي إلي تأثيرات شديدة علي هرمونات التناسل بالمقارنة بتحديد الغذاء لفترات طويلة.