Effects of Dietary Urea on timing of embryo cleavages and blood components in Mice

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Abstract

The objectives of the present study were to investigate the effects of dietary urea supplementation (1.0% and 3.0%) on oocytes quality, timing expected of embryo cleavages, offspring numbers and weights, blood components and rectal temperature in mice. Sixty of growing albino mice were classified into three groups; the control group was given basal control diet and the other two groups were fed on basal control diet supplemented with 1.0% and 3.0% urea. Body weights were recorded at the beginning and after 5 weeks. Thereafter, five female mice of each group were injected with 7.5 IU of pregnant mare serum gonadotropin (PMSG) for determination of oocyte quality after 48h of injection. The fifteen female mice of each group were injected with 7.5 IU of PMSG followed by 7.5 IU of human chorionic gonadotropin (hCG) after 48h and mated with males of proven fertility. Five mated females of each group were used for determination of embryo cleavages to four cell stage and the other five mated females were used for determination of embryo cleavages to eight cell stage upon 59-60 and 70 h of hCG injection, respectively. Rectal temperatures were recorded and blood samples were collected. The remaining five mated females of each group were left for parturition. The offspring number, litter size and male:female ratio were recorded. Hematocrit and hemoglobin concentrations were determined in blood whereas urea, total protein, albumin, glucose calcium and phosphorus concentrations were determined in plasma. The results indicated that offspring number and weight of litter size at birth were significantly ($P<0.05$) increased in the urea groups compared to control group. Percentage of good quality oocytes was high (70%) in control group compared to 3% urea group (60%). Dietary 3% urea was delayed cleavages to four-cell stage embryos at the expected time. Dietary urea was significantly ($P<0.05$) increased concentrations of hematocrit and hemoglobin in blood and urea, total protein, globulin, glucose, potassium and phosphorus in plasma. In conclusions, although 3% dietary urea decreased oocytes quality and timing expected of embryo cleavages to four cell stages, it increased significantly ($P<0.05$) offspring number and weight of litter size.

Key words: urea, germinal vesicle, cleavages, embryos

Introduction

Most previous reports have provided evidence that high protein diets, which result in elevated levels of plasma urea nitrogen, are related to decreased fertility in ruminants (McEvoy *et al.*, 1997; Dawuda *et al.*, 2002). The timing and mechanism(s) underlying the deleterious effects of excessive urea nitrogen on fertility remain unclear.

Studies have examined the effects of urea nitrogen on oocyte maturation and embryo development in vitro (De Wit *et al.*, 2001; Ocon and Hansen, 2003). The study of Rhoads *et al.*, (2006) demonstrated that the viability of the embryo is altered before day 7 of pregnancy in dairy cattle. High levels of plasma urea nitrogen changed the follicular, oviductal and (or) uterine environment, which impacted the competency of the embryos for continued development beyond day 7. In the present study, oocytes and embryos were recovered from superovulated donor mice fed diets supplemented with 1.0% and 3.0% urea. The collected oocytes and embryos were assessed for quality and stage of cleavages respectively.

In addition, offspring numbers and weights were recorded of the mated females. Blood samples were collected for analysis in addition to measuring rectal temperature.

The hypothesis that elevated plasma urea nitrogen concentrations would decrease embryo viability and result in fewer pregnancies.

Materials and methods

Animals and feeding: Sixty female albino mice (5 weeks of age) were used during the study for two months and classified into three groups. The control group was given basal control diet and the other two groups were fed on basal control diet supplemented

Table-1. Number of animals and composition of the experimental diets used during the study

Ingredients	Control	Urea 1%	Urea 3%
Corn, g	65.0	65.0	65.0
Soy bean, g	25.0	25.0	25.0
Mineral & vitamin mixture, g	10.0	10.0	10.0
Urea, g	0.0	1.0	3.0
Number of animals	20	20	20

Each 2.5 kg of mineral and vitamin mixture contains

Vit A	12.000.0000 IU	B2	5 gm	Folic acid	1 gm	Zinc	50 gm
Vit D3	2.000.000 IU	Vit B6	1.5 gm	Biotin	50 gm	Manganese	60 gm
Vit E	10 gm	Vit B12	10 gm	Choline chloride50%	250 gm	Iodine	1 gm
Vit K3	2 gm	Nicotinic acid	30 gm	Iron	30 gm	Selenium	0. 1 gm
Vit B1	1 gm	Pantothenic acid	10 gm	Copper	10 gm	Cobalt	0. 1 gm

Table-2. Effects of dietary urea level on body weight (BW), litter size and number and male / female ratio

Items	Treatments		
	Control	1% urea	3% urea
Initial body weight, g	14.18 ± 1.08	14.32 ± 1.66	14.28 ± 1.90
Mating BW, g	23.94^{a} ± 1.69	22.84^{b} ± 0.99	22.76^{b} ± 1.52
Litter size at birth, g	10.36b ± 1.85	12.64^{a} ± 0.35	12.88^{a} ± 0.52
Offspring number	7.6a ± 0.9	8.6a ± 0.54	8.8^{a} ± 0.45
Male/female ratio	19/19 (38)	21/22 (43)	22/22 (44)

a, b: Values with the different superscripts in the same row differ significantly (P<0.05).

with 1.0% and 3.0% urea. All mice were allowed free access to the experimental diets and water throughout the experimental period. Food and water were available ad libitum. Mice were killed by cervical dislocation for collection of oocytes and embryos. Composition of the experimental diets is shown in Table 1.

Body weight and rectal temperature : The body weights were recorded at the starting of experiment and before mating (after 5 weeks of starting). Weights of litter sizes were recorded upon deliveries. The body weight of animals was measured to the nearest ± 1.0 g using a standard balance. Rectal temperature was measured using a digital clinical thermometer.

Blood Samples : Blood samples were collected of ten female mice in each group before collection of cleaving embryos from the orbital sinus according to the method described by Hoff (2000). Hematocrit and hemoglobin concentrations were determined immediately in blood sample, then, the same remaining blood sample was centrifuged at 5000 rpm for 15 min for obtaining plasma and stored at -20 ℃ until further analysis.

Collections of germinal vesicle oocytes and embryos: Germinal vesicle (GV) oocytes were collected from the five matured female mice in each group. The female mice were injected with 7.5 IU of pregnant serum gonadotrophin (PMSG; Folligon, Intervet). Ovaries were removed from the donor females 44-48 h after PMSG injection. Antral follicles were punctured by 30-ga needles, and cumulus - GV oocyte complexes were released into Ringer's solutions supplemented with 10% serum. Germinal vesicle oocytes were aspirated immediately by glass pipette, the tip diameter of which was larger than the diameter of cumulus-enclosed oocytes and graded into good, fair and denuded oocytes.

Recording stage of cleaving embryos, weight of litter size and number and male/female ratio : Fifteen females of each groups were superovulated by injection of 7.5 IU of PMSG followed by 7.5 IU of human chorionic gonadotrophin (hCG; Chorulon, Intervet) 48 h later and mated with males of proven fertility. Ten females of each group were used for collection of cleaving embryos from the oviducts 59–60 and 70h h after hCG injection and the stages of embryos were recorded (Grabrek *et al*., 2004; Mohammed *et al*., 2010) whereas the remaining five females of each group were left for parturition. Weight of litter size and number and male/female ratio were recorded upon parturition.

Blood analysis : Blood samples were analyzed for determination of hematocrit and hemoglobin concentrations whereas plasma samples were analyzed for determination of urea, total protein, albumin, glucose, potassium and phosphorus concentrations by using appropriate commercial test kits supplied by Spectrum Diagnostics (Cairo, Egypt). The concentrations were measured using standard protocols.

Table-3. Effects of dietary urea level on quality of oocytes

Treatments	No. of Oocytes	Oocyte quality		
		Good	fair	Denuded
Control	50	70 (35/50)	20 (10/50)	10.0 (5/50)
Urea 1%	50	70 (35/50)	10.0 (5/50)	20 (10/50)
Urea 3%	50	60.0 (30/50)	20 (10/50)	20 (10/50)

Table 4. Effects of dietary urea level on timing of embryo cleavages collected 59–60 h and 70h after hCG injection

Treatments	No. of collected embryos	Expected four-cell stage embryos	
		Two cells% (n)	Four cells, % (n)
Control	40	12.5 (5/40)	87.5 (35/40)
Urea 1%	43	23.4 (10/43)	76.6 (33/43)
Urea 3%	45	26.6 (12/45)	73.4 (33/45)
		Expected eight-cell stage embryos	
Control	50	20.0 (10/50)	80.0 (40/50)
Urea 1%	45	17.7 (8/45)	82.3 (37/45)
Urea 3%	40	25.0 (10/40)	75.0 (30/40)

Statistical analysis: Data are presented as means ± SD. Differences between mean values were determined by ANOVA followed by comparisons using the Duncan's multiple range test. Differences with $P < 0.05$ were considered significant.

Results

Effects of dietary urea level on body weight gain, weights of litter sizes and numbers and male/female ratio are presented in table (2). Mating body weights (g) were significantly ($P < 0.05$) decreased in urea groups compared to control whereas weights of litter size at birth and offspring numbers were significantly ($P < 0.05$) increased in urea groups. Male to female ratio was not differed among groups. Percentage of good oocytes was decreased (60%) in urea (3%) group compared to urea (1%) and control (70%) groups (table 3). Furthermore, effects of dietary urea level on timing of embryo cleavages collected 59–60 h and 70h after hCG injection were presented in table (4). Urea levels (1 & 3%) were delayed timing of embryo cleavages at the expected time. The percentage of two cell embryos found at the expected four-cell stage embryos was higher (23.4 & 26.6%) in urea groups compared to control (12.5%) indicating delayed cleavages of embryos. The percentage of four cell embryos found at the expected eight-cell stage embryos was comparable among groups indicating restoring development. Rectal temperatures were not differed among groups (table 5). Dietary urea levels (1 and 3%) were significantly ($P<0.05$) increased hematocrit, hemoglobin, urea total protein, globulin, glucose, potassium and phosphorus. (table 5).

Discussion

Dietary urea levels (1 & 3%) were adversely affected on oocyte quality and delayed timing of embryo cleavages at four cell stages. Developmental competence of embryos was restored at eight cell stage. Thereafter, weights of litter size at birth and offspring numbers were significantly increased. Dietary urea supplementation was changed blood components where hematocrit, hemoglobin, urea, total protein, globulin, glucose, potassium and phosphorus concentrations were increased.

Mating body weights were significantly decreased in urea groups. Although the formulation of experimental diet supplied sufficient amounts of nitrogen, energy, minerals and vitamins (table 1) in urea groups and control group, this might be due to poor palatability of urea which reduced dry matter intake.

The adverse effects of dietary urea supplementation (1 & 3%) on oocyte quality in mice were consistent with our results in sheep (Mohammed *et al.*, unpublished). Moreover, dietary urea levels (1 & 3%) were associated with delayed embryo cleavages at the proper time. In other studies, dietary urea levels were associated with decreased embryo quality (McEvoy *et al.*, 1997). Thereafter, the embryos were restored developmental competence. Our results (Mohammed *et al*., 2005) concerning the effect of adding bovine follicular fluid to the maturation medium indicated that timing of first cleavage was delayed. In addition, developmental competence to the blastocyst stage was significantly increased compared to adding of serum.

Therefore, the changes of follicular fluid compositions might be responsible for the delayed timing of

Table-5. Effects of dietary urea level on rectal temperature and blood components

Items	Treatments		
	Control	1% urea	3% urea
Rectal temperature, ℃	36.46 ± 0.39	36.25 ± 0.44	36.61 ± 0.28
Hematocrit. %	55.0a ± 0.9	53.0b ± 2.4	56.0a ± 1.8
Hemoglobin, (g/dl)	9.2b ± 0.7	9.9a ± 0.5	10.3a ± 0.6
Urea, (mg/dl)	49.0b ± 4.8	53.4b ± 6.4	61.5a ± 9.5
Total protein, (g/dl)	7.16b ± 0.1	7.09b ± 0.26	7.48a ± 0.18
Albumin, (g/dl)	3.44 ± 0.07	3.48 ± 0.13	3.54 ± 0.11
Globulin, (g/dl)	3.6b ± 0.01	3.67b ± 0.13	3.93a ± 0.07
Glucose, (mg/dl)	127.4b ± 5.18	126.6b ± 14.81	145.4a ± 20.7
Potassium, (mg/dl)	7.27b ± 1.38	6.81b ± 0.30	8.27a ± 0.97
Phosphorus, (mg/dl)	5.75b ± 0.17	6.0a ± 0.23	6.1a ± 0.21

a, b: Values with the different superscripts in the same row differ significantly (P<0.05).

cleavages and increasing litter size at birth in urea groups. Considerable practical interest is the finding recorded by Dawuda *et al.* (2002) that cows are able to adapt within 10 days to the toxic effects of excess urea, and reduced fertility should not be evident beyond that time.

The increase in blood and plasma concentrations of hematocrit, hemoglobin, urea, total protein, globulin, glucose, potassium and phosphorus (Ziyadah *et al*., 2010) might be helpful for embryonic development as indicated by unchanged in rectal temperature. McEvoy *et al*., (1997) investigated the effect of dietary urea on the survival and metabolism of ovine embryos. They fed ewes a maintenance diet with no urea (control) or with urea at 15 g (low urea, LU) or 30 g (high urea, HU) kg- 1 feed for a 12 week period. Day 4 HU embryos were retarded relative to C and LU embryos. McEvoy *et al*., (1997) concluded that excess rumen degradable N in ewe diets elevates urea and ammonia in plasma and in uteri, with an associated increase in embryo mortality. Nevertheless, metabolism appears to be up-regulated in some embryos and, among those that survive, fetal growth appears to be enhanced. The possible physiological negative effects of dietary urea on folliculogenesis and embryo development might be due to elevated blood urea nitrogen and consequently increased ammonia N concentrations in follicular fluid. Hammon *et al*. (1997) found that ammonia N concentrations in follicular fluid are negatively associated with ovarian follicle size.

Furthermore, dietary urea supplementation decreased ions concentrations of plasma in this study. Wise, (1987) suggested that the markedly higher Ca concentration in follicular fluid during the earlier stages of the cycle may play a crucial role in steroidogenic capability of the growing follicle which may negatively decrease with dietary urea supplementation.

In conclusion, dietary urea supplementation affects on fertility through the period of folliculogenesis and preimplantation embryonic development.

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