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Embryo development and follicular status of Toggenburg does fed urea diet[1]

Lincoln da Silva Amorim[2], Ciro Alexandre Alves Torres[2], Luiz Gustavo Bruno Siqueira[3], Jeferson Ferreira da Fonseca[4], José Domingos Guimarães[2], Giovanni Ribeiro de Carvalho[2], Nadja Gomes Alves[5], Margarida Maria Nascimento Figueiredo de Oliveira[6]

[1] Pesquisa financiada pelo CNPq e pela FAPEMIG.
[2] Universidade Federal de Viçosa.
[3] Embrapa Gado de Leite.
[4] Embrapa Caprinos e Ovinos.
[5] Universidade Federal de Lavras.
[6] Universidade Federal do Vale do Jequitinhonha e Mucuri.

ABSTRACT - The effects of feeding urea on embryo production, quality and developmental stages and follicular status at the beginning of a superovulatory treatment of Toggenburg does fed urea diet were investigated in this study. Eighteen females were randomly allocated to receive diets with: no urea (control, n=8), and 2.4% urea dietary dry matter (n=10) UDM. The embryo recovery was performed at day 7 or 8 of estrus cycle by transcervical technique and classified according to quality and developmental stage. The follicular status was determined by transrectal ultrasonography at the first day of FSH injection. The number and quality of embryos were not affected by dietary urea concentration. Embryos from does treated with 2.4% UDM were recovered at advanced stage. Urea concentration (2.4%) decreases the number of follicles with less than 5 mm diameter. This follicle class is positively correlated to the number of recovered embryos and to the number and percentage of excellent and good embryos.

Key Words: embryo, follicle, goat, superovulation, urea

Desenvolvimento embrionário e *status* folicular de cabras Toggenburg alimentadas com dieta à base de ureia

RESUMO - Objetivou-se investigar os efeitos da ureia na produção, qualidade e estádio de desenvolvimento embrionário e *status* folicular ao início do tratamento superovulatório de animais alimentados com ureia na dieta. Dezoito cabras da raça Toggenburg foram distribuídas equitativamente para receberem dietas: sem ureia (controle, n=8) e 2,4% de ureia na matéria seca total da dieta (n=10) UMS. Os embriões foram recuperados entre o 7º o 8º dia após o estro pela técnica transcervical e classificados pela qualidade e pelo estádio de desenvolvimento. O *status* folicular foi determinado por ultrassonografia transretal no dia da aplicação da primeira dose de FSH. O número e a qualidade dos embriões não foram influenciados pela concentração de ureia na dieta. Embriões em estádio avançado de desenvolvimento foram recuperados do tratamento com 2,4% de ureia. A concentração de ureia testada (2,4%) diminui o número de folículos com menos de 5 mm de diâmetro, categoria que se correlaciona positivamente ao número de embriões coletados e ao número e à porcentagem de embriões excelentes e bons.

Palavras-chave: caprinos, embrião, folículo, superovulação, ureia

Introduction

Urea and ammonia are biochemical compounds that may affect the development of mammal embryos. Based on *in vivo* or *in vitro* trials, ammonia and urea produced by cellular metabolism and the catabolism of amino acids *in vitro* or *in vivo* by adding urea or the exceeding dietary protein and its digestion are toxic to the sheep embryo and gametes (McEvoy et al., 1997), cattle (Hammon et al., 2000a,b; Ferreira, 2007; Alves, 2007) and goats (Alves et al., 2007). Oocyte maturation, fertilization and the beginning of embryo development are all affected by the micro-environment, which is affected by the protein ingestion (Jordan et al., 1983; Elrod & Butler, 1993).

Feeding diets with excess of protein or urea alter the biochemicals profile of the blood, which can affect the uterus, oviduct and follicle environments (Amorim, 2008). Therefore, the normal development of the embryo may be affected.

Reduced viability, gene expression changes, impairment of cellular structures, changes on *in vivo* and *in vitro* development and on intrauterine migration are the main consequences of the adverse effects on embryos.

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Corresponding author: lnlsamorimufv@gmail.com

It has been postulated that embryos are able to regulate the amount of urea required for their metabolism and growth. However, few studies have been conducted to effectively evaluate the ability of metabolic exchange with their environment.

This paper assessed the effect of feeding a control diet and a diet with 2.4% urea (total DM basis) on nutrient intake, number of recovered structures and embryos, embryo quality and developmental stage and follicular status of Toggenburg does at the beginning of FSH treatment.

Material and Methods

This trial was carried out from May to July 2006 during natural breeding season of goats (Autumn in the Southern Hemisphere), in the Água Limpa Goat Farm, situated in the Piau county in Southeast Brazil. This region is characterized by a CWa climate (21º 35' S, 43º 15'), according to Köppen-Geiger classification (dry winter and wet summer).

Eighteen Toggenburg does, *Capra hircus* (Linnaeus, 1758), selected by general clinical examination and good health history, free of diseases and with good body condition score: 2.9 ± 0.5 in a scale of 0 – 5 (0 being very thin and 5 being fat) (Villaquiran et al., 2005) were used. The multiparous does had live and healthy kids and the ovaries (follicular growth), the uterus (with/without hydrometra) and the presence of other uterine and ovarian pathologies, were evaluated by ultrasound (Aloka, SSD 500 – Japan) once a week for at least two consecutive regular estrous cycles. The selected females were nulliparous, primiparous and multiparous, not pregnant, not lactating, with 48.6 ± 7.9 kg and 34.3 ± 20.8 months old at the beginning of the experiment.

Females were equally assigned to 2 groups according to the weight, age and body condition score: control (n=8) and treatment with 2.4% urea (n=10). Animals were housed in individual pens (1.5 m^2) with collective drinkers and individual feeder.

The animals gradually received a diet with 2.4% urea (day 0: beginning of the treatment with 2.4% urea, Figure 1) and, every week before the beginning of the experiment, they received diets with three urea levels (0.0, 1.0 and 2.0%) to adapt to the diet.

Diets comprised Tifton 85 (*Cynodon* spp) hay and concentrate and were formulated to be isonitrogenous (14% CP/kg DM) and isocaloric (1.4 Mcal/kg DM). Non-protein nitrogen from urea (in relation to total protein) were 0.00, 13.00, 26.00 and 39.25% and non degradable protein were 59.13, 63.23, 67.07 and 71.18 for diets with 0.0, 1.0, 2.0 and 2.4% of urea (Table 1).

Table 1 - Composition of the experimental diet (DM basis)

	Diet (%urea)			
Feedstuff	0	1.0	2.0	2.4
Tifton 85 hay	52	53	52	53
Corn meal	4	9	12	22.5
Soybean meal	12	7	3	0
Wheat bran	27	27	27	18.2
Bicarbonate	1	1	1	1
Nucleus*	1	1	1	1
Urea	0	1	2	2.4
Limestone	3	3	3	2.3

* Calcium - 140 g; phosphorus - 70 g; sulfur - 10 g; magnesium - 10 g; sodium - 125 g; iron - 675 mg; cupper - 875 mg; zinc - 2.700 mg; manganese - 1.750 mg; chrome - 6 mg; iodine - 46 mg; selenium - 15 mg; cobalt - 46 mg; vit. A - 150 mg; vit. D - 60 mg; vit. E - 300 mg; fluorine - 700 mg.

The experimental diets were provided twice a day (07:30 am and 04:30 pm), being adjusted daily according to the consumption of the previous day, allowing leftovers of approximately 5% of total from dry matter provided. Animals had free access to water and mineral salt. Leftovers were daily collected before the morning feeding and weighed for intake analysis.

The leftovers (10%) were daily sampled and frozen (-20ºC). At the end of this period, a composite sample was made for week within each animal. All samples (leftovers and food) were thawed, grounded using a Wiley mill with a 1 mm screen, packed in glass bags, labeled and frozen for subsequent chemical analysis (Table 2). Feed samples were analyzed for dry matter, total nitrogen, ethereal extract and ash (Silva & Queiroz, 2002) and also for neutral acid detergent fibers and lignin in acid detergent (Van Soest et al. 1991). Total carbohydrates (CHOT) were calculated as CHOT: 100 – (CP% + EE% + Ash%) (Sniffen et al., 1992) and nonfiber carbohydrates (NFC) as TDN = DCP + 2.25 × DEE + DTC, in which DCP = digestible protein, DEE = digestible ether extract and DTC = digestible total carbohydrates.

Synchronization and superovulation protocols followed the procedures recommended by Alves (2005), with adaptations. The following protocol was performed only once in each animal, thus the embryos collection after receiving the experimental diets also happened once. Intravaginal sponges immersed in 60 mg of medroxyprogesterone acetate were applied (Progespon® - Sintex S.A., Tecnopec LTDA, SP, Brazil) for 11 days, coinciding with the day 7 of feeding diet with 2.4% urea. Superovulation protocol began 48 hours before intravaginal sponge removal and was carry out by the injection of 200 mg of NIH-FSH-PI (Folltropin® - Bioniche) in six decreasing doses, with 12-h interval between applications (50, 50, 30, 30, 20 and 20 mg). Simultaneously with the FSH injection, 100 µg of sodium cloprostenol was injected

Table 2 - Chemical composition of feedstuffs

	Feedstuff			
	Tifton 85 hay	Corn meal	Wheat bran	Soybean meal
Dry matter (%)	91.75	86.94	87.15	88.35
Crude protein (% DM)	6.53	7.19	13.81	48.78
Ethereal extract (% DM)	0.86	3.55	3.38	2.09
Lignin (% DM)	6.16	1.16	4.0	1.33
Neutral detergent fiber (% DM)	70.28	20.54	37.81	12.55
Acid detergent fiber (% DM)	39.79	4.08	13.52	9.86
Total carbohydrates (% DM)	87.49	87.89	77.27	42.67
Total digestible nutrients (% DM)	55.62	87.24	72.43	81.54
Ash	5.12	1.38	5.54	6.47
Calcium	0.42	0.03	0.22	0.34
Phosphorus	0.17	0.25	1.00	0.58

(Ciosin® - Schering Plough Veterinária) intramuscularly. Seventy-two hours after sponge removal, the antiluteolytic treatment began with 1.1 mg/kg/d of flunixin meglumine (Banamine® - Schering Plough Veterinária) during three consecutive days (Figure 1).

Ultrasound examinations of the ovaries were done to determine the follicular status at the beginning of superovulatory treatment using an ultrasound (ALOKA SSD 500) with 5.0 MHz linear transducer coupled to a rigid extension. After been restrained at standing position in a commercial squeeze chute, the animal feces were collected and a carboxymethyl cellulose gel (Carbogel ULT®) was added for better visualization of ovarian structures. Ultra sound examinations were performed after the synchronization protocols in a coincident date with the beginning of the superovulation or during the first FSH injection in order to assess ovarian activity. Both ovaries were examined, and the number, diameter and location of all antral follicles ≥ 3 mm were recorded. Follicles were classified as small, medium or large (3 to 4 mm, >4 to <5 mm and ≥5 mm of diameter, respectively) according to Menchaca et al. (2002) recommendations.

From 12 hours after sponge removal, females were monitored for estrus twice (7:00 am and 7:00 pm) for at least 15 minutes using a surgically prepared buck. Does in estrus were bred by fertile Toggenburg bucks every 12 hours until mounting was refused.

Embryos were recovered by transcervical technique in closed circuit, between day 7 and 8 of estrus cycle (estrus = day 0). Twenty-four hours before the expected time for embryo recovery, all females received an injection of 125 µg of sodium cloprostenol intramuscularly. Embryos were recovered while the animal was restrained in the squeeze chute. After shaving the tail and perineal hygiene, each does that would be submitted to embryos recovery received 1% acepromazine (Acepran® - Univet S.A.) in a proportion of 1 mL/100 kg BW intramuscularly (preanesthetic) and 2 mL/animal of lidocaine hydrochloride 2% without

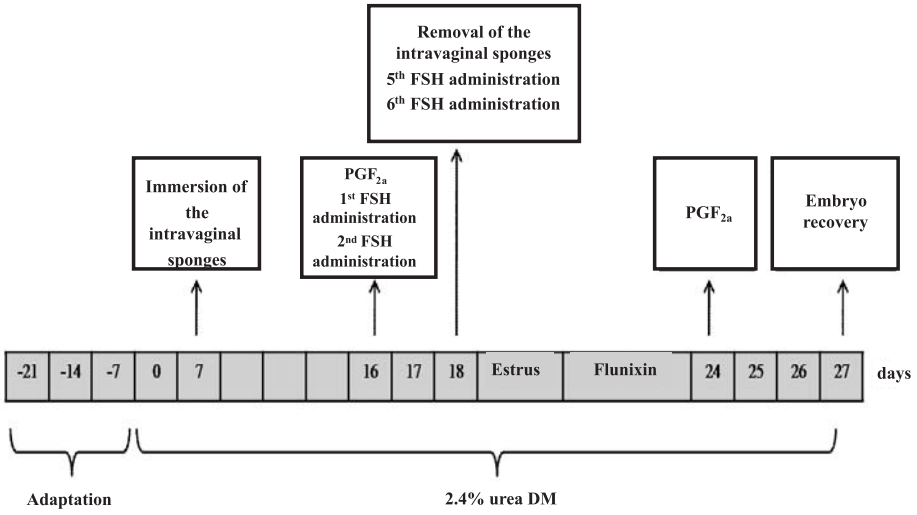


Figure 1 - Superovulation protocol of goats fed urea diet.

vasoconstrictor (Lidovet® - Bravet), by epidural via in the sacrococcygeal region. Cervix was examined with a vaginal speculum and a light source. A sterile gauze soaked with 2 mL lidocaine 2% (Lidovet® - Bravet) was introduced and kept in contact with vaginal fornix for 30 seconds. Following this procedure, the cervix was pinched and lightly pulled with two Pozzi tweezers towards the vagina vestibule. Then, a sterile urethral catheter 10 or 12 (Fabrimed® - Fabrimed) equipped with a metal mandrel (which was removed after the passage of the rings) was used to bypass the cervical rings.

A circuit for collecting embryos in a fully closed system consisting of transparent, non-toxic, sterile and flexible vinyl tubes (BD® - BD) was assembled to allow two ways of communication with the urethral catheter and both were connected to the probe in Y (Cremer® - Cremer S.A). One of the circuit lines communicated with the collection filter of the embryos and the other was connected to the hose linked to the washing medium (Modified Dulbeco DPBS - Embriocare® - Cultilab) at 37 ºC. In the last portion of the filter, a 50 mL syringe (BD ®) was placed immediately before the communication with the washing medium, to control the volume of liquid instilled in each uterine horn, which was approximately of 200 mL.

In order to wash each uterine horn with the embryos collection medium, the route attached to the filter for embryo collection was closed by a flow obstruction (Cremer ®) and the route linked to a urethral catheter was opened, so that the washing medium was instilled in each uterine horn. Following this procedure, the collection medium was kept for a few minutes inside the uterus, and then the route attached to the filter was opened for embryo recovery. Approximately 50 mL were instilled in each uterine horn, one at a time, until 200 mL were completed.

The uterine washings were filtered after being recovered and its contents were prepared in Petri dishes (100 x 20) and screened for the identification of embryos under a stereomicroscope at 10X magnification (Olympus®). Immediately after identification, the samples were transferred to smaller Petri dishes for evaluation of quality and developmental stage, at 40X magnification.

The total number of structures, embryos and oocytes and the quality and developmental stage of recovered embryos were determined for each group. A quality grade (1 - being excellent and 5 - being degenerated) was assigned to each of the recovered embryos, following the recommendations of Stringfellow & Seidel (1999) who consider viable the embryos with degrees 1 (excellent), 2 (good) and 3 (regular). The developmental stages determined were: oocytes, morula, compact morula, early blastocyst, blastocyst, expanded blastocyst, hatched blastocyst and degenerated one.

Analysis of variance (ANOVA) were performed utilizing the software *Sistema de Análises Estatísticas e Genéticas – SAEG 9.0* (UFV, 2003) using a 5% level of significance. Comparisons of averages were made using the Tukey test. The Cochran and Bartlett tests were employed for testing the homogeneity of variances for the number of structures, embryo, viable embryo and excellent and good embryos, percentage of viable embryo and excellent and good embryos, parameters of ovary at the FSH injection, total number of small, medium and large follicles and the largest diameter follicle. The normality and homoscedacity of the results distribution was tested according to the Lilliefors test. The embryo developmental stage characteristics are presented as descriptive statistics. Pearson correlations were performed for embryo parameters and follicle status at the first FSH injection.

Results and Discussion

The urea in the diet did not affect the individual mean intake of the diet nutrients (Table 3). As the diets were isoenergetics and isoproteics and differed only for non-protein nitrogen and ruminal degradable protein quantities, the feed intake was the same for both groups. The constituents and proportion of roughage used probable increased the ruminal ammonia concentration in the group fed 2.4% urea diet but did not alter the nutrient intake.

Reduced DM intake has been reported in beef heifers fed 240 g/d of urea (Kenny et al., 2002) and in cows fed high amounts of non-protein nitrogen (Conrad et al., 1977). Similarly, Sinclair et al. (2000) reported reduced DM intake in heifers fed high plasma ammonia concentration and Oliveira (2001) also observed reduced DM intake as the dietary urea concentration increased up to 2.4% on total DM basis. However, Alves (2005) observed increasing intake of dry matter, crude protein, ethereal extract, organic matter and total digestible nutrients in Alpine goats fed urea diets (0.0, 0.7, 1.4 and 2.24% DM) using corn silage as roughage.

Table 3 - Mean individual nutrient intake from goats fed urea diet

	Urea concentration (%DM)	
	0	2.4
Dry matter (kg/d)	1.05 ± 0.15	0.99 ± 0.25
Crude protein (kg/d)	0.08 ± 0.01	0.08 ± 0.02
Ethereal extract (kg/d)	0.009 ± 0.001	0.009 ± 0.002
Neutral detergent fiber (kg/d)	0.33 ± 0.05	0.31 ± 0.08
Total digestible nutrients (kg/d)	0.35 ± 0.05	0.33 ± 0.083
Carbohydrates (kg/d)	0.46 ± 0.07	0.44 ± 0.11

(P>0.05).

Singhal & Mudga (1980) and Fernandez et al. (1997) reported that urea can be supplemented in amounts exceeding those recommended by NRC (1981), 1/3 of the total crude protein or up to 50% of concentrate protein, because it does not alter the intake.

Most does (16/18) manifested estrus after synchronization. No treatment effect (P>0.05) on estrus duration (hours), interval from sponge removal to the start of estrus (hours) and interval from sponge removal to the end of estrus (hours; Table 4). Similarly, Fahey et al. (2001) and Alves et al. (2007) reported no treatment (urea) effect on estrus parameters of ewes and does (50 g/d or 0.0, 0.7, 1.4 and 2.24% of total DM basis, respectively).

Estrus synchronization protocols, similar to the one used in this study, in Moxotó goats (Andrioli et al., 2000), crossbred goats (Andriolo-Pinheiro et al., 1996) and Alpine goats (Alves et al., 2007), resulted in 80.0, 60.9 and 81.2% of estrus, respectively, values very close to the ones observed in this study. The estrus duration in animals fed 0.0 and 2.24% of urea was 27.6 and 30.3 hours (Alves et al., 2007), respectively, values similar to the ones found in this study (28.8 and 22.8 for 0.0 and 2.24% of urea, respectively).

Six does produced no structure or embryo (n=3, 0% and n=3, 2.4% of urea) from a total of 18 females that manifested estrus and were submitted to embryo recovery. Twelve does (n=5, 0% and n=7, 2.4% of urea) were responsive to the superovulation protocol, producing 80 structures (75 embryos and 5 oocytes). Thirty-six embryos (48%) were recovered in the control group and 39 embryos (52%) in does fed 2.4% of urea, therefore no difference was detected between treatments (P>0.05; Table 5). The number of non fertilized oocytes differed (P<0.05) between groups (0.0 and 2.4% urea; Table 5).

Reduced percentages of fertilized oocytes of the total recovered structures in females fed diets with high ruminal non-degradable protein have been reported in studies with superovulated cows (Blanchard et al., 1990) and ewes (Bishonga et al., 1996). Similarly, Alves (2007) reported lower fertilization capacity of oocytes in cows daily fed 100 g urea/animal after superovulation in relation to the group that received urea before superovulation. Urea can be toxic to cattle gametes and result in infertility, because ammonia from urea metabolism can increase mortality of sperm and oocytes (Visek, 1984). Dasgupta et al. (1970) (quoted by Blanchard et al., 1990) observed that at high concentrations urea is harmful to human and rat sperm and decrease the ability of sperm to penetrate in vitro cattle cervical mucus (Blanchard et al., 1990).

Table 4 - Characteristics of synchronized estrus in does fed urea diets

	Urea concentration (%DM)	
	0	2.4
Animals in estrus (%)	100 (n=8)	80 (n=8)
Estrus duration (hours)	28.8 ± 14.8	22.8 ± 15.4
Interval from sponge removal to the start of estrus (hours)	31.4 ± 12.4	28.5 ± 18.2
Interval from sponge removal to the end of estrus (hours)	60.2 ± 22.9	51.3 ± 28.8

P>0.05.

Table 5 - Embryo production of does fed urea diet

	Urea concentration (%DM)	
	0	2.4
Oocytes Number	0b	5a
Fertilized/recovered, %	100	88.64
Number of recovered embryos	36	39
Average number of embryo per does	7.2	5.5
Average number of viable embryo (1, 2 and 3)	7.0 ± 2.12	5.4 ± 3.3
Viable embryos, %	100	84.41
Average number of excellent and good embryos (1 and 2)	6.80 ± 2.49	5.11 ± 3.44
Excellent and good embryos, %	95	65

Means within a row with different letters differ (5%) by Tukey test.

No treatment effect (P>0.05) on the total and average number of embryos, number of viable embryo and percentage of viable embryo was observed (Table 5).

Many authors have reported that the number of recovered embryos is not likely due to the excess of urea diets or ruminal degradable protein for cows (Blanchard et al., 1990; Alves, 2007), heifers (Gath et al., 1999), sheep (McEvoy et al., 1997; Fahey et al., 2001; Papadopoulos et al., 2001) and goats (Alves et al., 2007). According to Alves et al. (2007) the average number of recovered embryos per donor by transcervical technique was 8.5 from Alpine does fed urea diets (0.0, 0.73, 1.46 and 2.24%). Androukovitch et al. (2002) recovered 13.2 embryos from Boer donors by transcervical technique with human urethral catheter. Other authors reported an average recovered embryos of 8.4 from Boer (Gusmão et al., 2003) and for undefined breed goats (Salles et al., 2003), of 7.6 from Saanen goats (Pereira et al., 1991) and of 6.3 from Saanen goats (Lima-Verde et al., 2003).

Deleterious effects on embryos quality of cows, ewes and dams fed urea diets or diet with excess of ruminal degradable protein has been reported. Although urea reduces the number and percentage of viable embryos in goats, the quadratic effect of regression curves as a function of urea intermediary concentration showed that embryos from dams fed 2.24% urea were similar to the control group (0%) (Alves et al., 2007). Feeding urea (100 g/animal/d)

either before or after superovulation may decrease the embryo viability of cows (Alves, 2007). Some studies reported that animals previously adapted to high urea concentrations seems to have no effects on embryo quality, because they can adapt to the toxic effect from urea diets. However, the duration of the exposure to ammonia, the ammonia concentration and the developmental stage to which the embryos are exposed affect in vitro development of embryos (Hammon et al., 2000a, b).

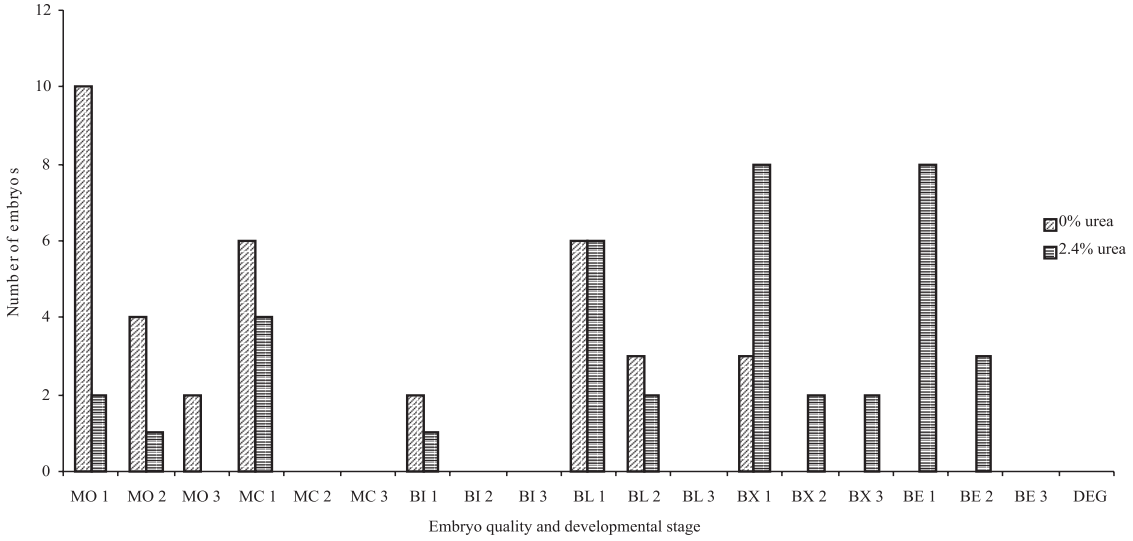
Blanchard et al. (1990) reported that the number of recovered transferable (excellent, good and regular) and not transferable embryos from cows fed 73 and 64% rumminal degradable protein were similar. Also, Berardinelli et al. (2001) feeding dams with 100 or 200% of the protein required for maintenance, did not detected differences among embryos, recovered at different days after mating. No difference was found in the embryo quality of cows fed 250 g/animal/d in relation to the control ones for a long period prior to insemination (Dawuda et al., 2002) and in the number of transferable embryos of cows fed 14 or 18% CP in the diet (Mikkola et al., 2005).

However, the visual quality of the embryos evaluated through stereomicroscope may not reflect whether these embryos would be able to develop normally if they were transferred. Embryos from donors fed in excess of urea diet showed high embryo mortality when transferred to receptors fed free-urea diet and presented a differential pattern of some genes expression related to the metabolism and the control of development, as IGF2R (Powell et al., 2006). Embryos with reduced developmental ability after transfer process from dams fed diets with different CP levels produced different plasma urea nitrogen concentration (Rhoads et al., 2006).

Alves (2005) selected embryos from does fed three urea levels (0.0, 0.7 and 1.4%), which were classified as excellent, but, reported that the transmission electron microscopy revealed ultrastructural imperfections in the embryos. Main changes included increasing morphological structures due to the process of reorganization and/or cellular degeneration, which increased as the dietary urea concentration increased.

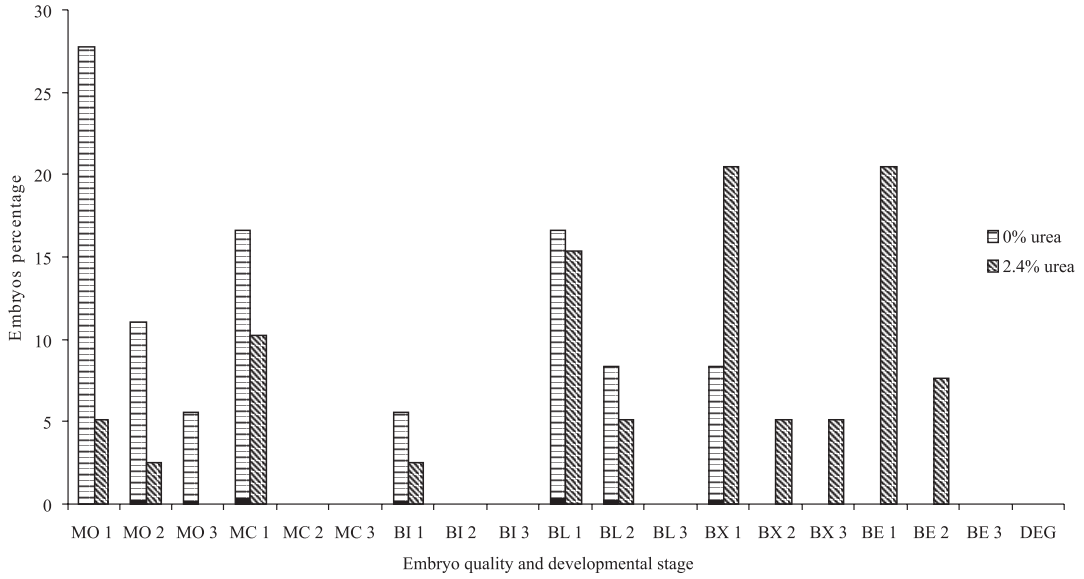
The following developmental stages were recovered: 19 morulas (25.33%), 10 compact morulas (13.33%), 3 initial blastocysts (4%), 17 blastocysts (22.66%), 15 expanded blastocysts (20%), 11 hatched blastocysts (14.66%). Sixteen morulas (44.44%), 6 compact morulas (16.66%), 2 initial blastocysts (5.55%), 9 blastocysts (25%) and 3 expanded blastocysts (8.33%) were recovered in animals fed 0% urea (Figures 2 and 3), 3 morulas (7,69%), 4 compact morulas (10.25%), 1 initial blastocyst (2.56%), 8 blastocysts (20,51%), 12 expanded blastocysts (30.76%) and 11 hatched blastocysts (28.20%) were recovered in those fed 2.4% urea (Figures 2 and 3).

Embryo developmental stage may vary among animals whose embryo collection is performed on the day of estrus and among embryos recovered from the same does (Baril et al., 1981, quoted by Gordon, 1997). But, feeding urea (2.24%) to Alpine goats resulted in high percentage of hatched blastocysts (Alves et al., 2007), the same result observed with high percentage of expanded and hatched blastocysts collected from Toggenburg does.



MO: morula; MC: compact morula; BI: early blastocyst; BL: blastocyst; BX: expanded blastocyst; BE: hatched blastocyst; DEG: degenerated; 1: excellent embryo; 2: good embryo; 3: regular embryo.

Figure 2 - Number, quality and developmental stages of embryos from Toggenburg does fed urea diet.



MO: morula; MC: compact morula; BI: early blastocyst; BL: blastocyst; BX: expanded blastocyst; BE: hatched blastocyst; DEG: degenerated; 1: excellent embryo; 2: good embryo; 3: regular embryo.

Figure 3 - Percentage, quality and developmental stages of embryos of Toggenburg does fed urea diet.

Number and proportion of embryos in the advanced stage (expanded and hatched blastocysts) from animals fed 2.4% urea diet were greater than the control group (without urea). This is supported by the finding of McEvoy et al. (1997) who observed embryos in advanced stage from dams fed 30 g urea/kg in the diet in relation to those fed 2.5 g urea/kg diet. Higher proportion of embryos in advanced development stage from dams fed urea diet (2.24% DM) were observed, suggesting a stimulatory effect of urea on the embryo development (Alves et al., 2007). Also, Berardinelli et al. (2001) found greater number of cells in sheep embryos fed 200% of protein required for maintenance in relation to those fed 100% at the fifth day after mating (Berardinelli et al., 2001). Especific estrogen-dependent glycoprotein secreted by the oviduct and also present in the zonna pellucida and in the perivitelline space may have stimulatory effect on the embryo development (Berardinelli et al., 2001). It is likely that urea can affect at least one of these mechanisms and accelerate the embryo development or even the reprogrammation of embryo exposed either to high ammonia or urea concentrations in the start of development. Similarly, zygotes were recovered at an advanced stage from donors fed 30 g urea which showed high plasma urea concentrations. This fact is likely due to a reduced expression of gene type-II insulin-like growth factor receptor (IGF2R), an imprinted gene associated with loss of methylation of the second intron of the differentially methylated region. This gene was related to the development of embryos with the syndrome large weight offspring (Powell et al., 2006).

The diets did not affect the ovarian characteristics ($P>0.05$) before the first FSH injection (Table 6), but the number of follicles ≥ 5 mm ($P<0.05$). Low number of large follicles ($P<0.05$) was observed for goats fed urea diet. Considering that this is the first study to evaluate the follicle status before superovulation in does fed urea diets, more studies are needed to validate these results.

The number of large follicles was positively correlated to the number of recovered embryos ($r=0.33$, $P=0.14$), number of viable embryos ($r=0.33$, $P=0.14$), number of excellent and good embryos ($r=0.34$, $P=0.15$) and percentage of excellent and good embryos ($r=0.43$, $P<0.10$). Gonzales-Bulnes et al. (2003) also observed positive correlation between the number of 4-6 mm follicles and the number of recovered and viable embryos in goat. The lack of association between the number of 2-3 mm follicles and the

Table 6 - Follicle status before the first FSH injection in Toggenburg does fed urea diet

	Urea concentration (%DM)	
Number of follicles in each diameter class	0	2.4
≥ 3 and < 4 mm (small)	5.9 ± 3.9	4.2 ± 3.5
≤ 4 and > 5 mm (medium)	1.6 ± 0.7	1.4 ± 1.3
≥ 5 mm (large)	2.5 ± 2.0a	1.1 ± 1.0b
Larger follicle (mm)	0.6 ± 0.6	0.6 ± 0.1

Means with different letters in a row differ at 5% by Tukey test.

number of recovered embryos was also described by Gonzales-Bulnes et al. (2003) in does, indicating that small follicles grow up to the pre-ovulatory stage, but are not able to produce one viable oocyte. Probably these follicles would be in the initial stage of atresia or immature. Besides the urea seems to affect on its growth, because the number of these follicles was low in does fed urea (Table 6).

Conclusions

Feeding 2.4% of urea (dry matter basis) alters neither the dry matter intake nor the number and quality of recovered embryos of nonlactating Toggenburg does. Dietary urea concentration might affect the embryo developmental stage. Large follicles are reduced when the animal is supplemented with urea. The follicle status before the first FSH injection positively correlates to the number of recovered embryo as well as the number and percentage of excellent and good embryos.

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