

Metabolic profile of pregnant, non-pregnant and male two-humped camels (*Camelus bactrianus*) of Iran

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Abstract:

BACKGROUND: The knowledge in blood constituents is important for assessing the physiological status and the health of animals. Only a limited number of two-humped camels (*Camelus bactrianus*) were reared in Ardabil province, northwest of Iran. **OBJECTIVES:** The present study was carried out to find out whether the sex and pregnancy affect thyroid hormones, lipid and lipoprotein profile, and selected biochemical factors in healthy Iranian Bactrian camels. **METHODS:** The evaluated herd contained twenty clinical healthy Bactrian camels, aged between four and thirteen years. Six of them were non-pregnant, five in the late period of pregnancy, and nine were male camels. Blood samples (10 mL) were collected from the jugular vein of camels in spring 2013 during two consecutive days. All samples were centrifuged at 3000× rpm for 15 min and sera were refrigerated at -21°C until analysis. Thyroid function tests were carried out by measuring serum levels of thyroid stimulating hormone (TSH), free and total thyroxin (fT4), (T4), and free and total triiodothyronine (fT3), (T3) by commercially available radio immunoassay kits. The biochemical parameters were measured using a standard autoanalyser (Hitachi717, Boehringer. Mannheim, Germany). **RESULTS:** The levels of fT4, HDL-Cholesterol, ALP, and glucose in the sera of pregnant camels were significantly lower than the male and non-pregnant camels. The serum levels of other parameters were not significantly different among the groups. **CONCLUSIONS:** The present study revealed that heavy pregnancy has a profound effect on certain serum biochemical parameters in Bactrian camels.

Introduction

In the central parts of Asia, there are two-humped camels, known as the bactrian. Bactrian camels endure temperature extremes, from -40 degrees in winter to +40 in the summer and can survive up to three days without food or water and up to one week in very cold weather. Camels are sexually mature when they are four or five. Mating takes place from December to February. After gestation for 12 to 15 months, females give birth to a single calf weighing

up to 45 kg. Calves can stand soon after birth, are weaned at one to two years, and stay with the mother until they reach maturity. The life span of the domestic bactrian camel is approximately 40 years. Bactrian camels are shorter and heavier than the one-humped dromedary. The domesticated bactrian camel is widely used in central Asia and western China because of its adaptation to harsh climates (Zongping, 2003). Only a limited number of two-humped camels (*Camelus bactrianus*) were reared in Iran, and most of them were kept at Jahanabad

breeding center of Meshkinshahr in Ardabil province, northwest of Iran (Niasari-Naslaji et al., 2008). Our present knowledge about nutrition, physiology, and genetics of bactrian camel is limited because little research has been done. Compared to other domestic animals such as dairy cattle, sheep, and goats, our understanding about the physiological and hormonal changes that the camel undergoes during pregnancy is inadequate. Because of the long pregnancy period of camels, it was assumed that energy requirements of pregnant camels increase rapidly during the heavy pregnancy. This may affect the concentration of some biochemical parameters. Also, during pregnancy some metabolic changes occur that may alter blood constituents (El-Sherif and Assad, 2001; Khan and Ludri, 2002). Pregnancy is a dynamic process characterized by dramatic physiological changes that may influence hormonal functions and biochemical values in the animal. Thyroid function regulates a wide range of metabolic activities (Aziz khan et al., 2014). Appropriate thyroid gland function and its hormonal activity are crucial to sustain the reproductive performance, healthy pregnancy outcomes, and successful brain development in the fetus of animals (LaFranchi et al., 2005). Thyroid function significantly affects lipoprotein metabolism (Duntas, 2002; Eshratkhah and Sadaghian, 2010). The liver metabolizes lipids. Lipids play an important role in the pregnancy. They serve as hormones or hormone precursors, provide energy, and act as structural components in the cell membranes. Many researchers evaluated the normal concentrations of serum lipids and lipoproteins of the sheep (Eshratkhah and Sadaghian, 2010; Nazifi et al., 2002a), goats (Nazifi et al., 2002b), ewes (Piccione et al., 2009), cows (Mohebbi-Fani et al., 2012a; Mohebbi-Fani et al., 2012b), horses (Nazifi et al., 2005), pony mares (Watson et al., 1993), camel (Nazifi et al., 2000; Asadi et al., 2009), and women (An-Na et al., 1995). During pregnancy, blood serum constituents may be influenced by several factors such as breed, age, malnutrition, or season. Also, maternal tissues are involved in providing energy for reproduction processes, and growth of the fetus (Swanson et al., 2004; Yokus et al., 2006). In sheep, during the late pregnancy, the blood serum lipid profile is characterized by increased concentration of total cholesterol, triglycerides, and lipoproteins (Schlumbohm et al.,

1997). Variation in blood cholesterol content has been observed during pregnancy. Cholesterol is the precursor of the steroid hormones (Iriadam, 2007). Lipid profiles have been used to predict peripartum diseases (Nazifi et al., 2002b). The liver also plays an essential role in carbohydrates and amino acid metabolism (Ouajd and Kamel, 2009). Measurement of some blood constituents and enzymes (aspartate amino transferase concentrations, alanine amino transferase, alkalinephosphatase, and gammaglutamyl-transferase) need for evaluation of the health of the liver. Under normal circumstances, these enzymes exist within the hepatocytes; however, when the liver is injured, these enzymes enter the blood stream (Yap and Choon, 2010). Total protein and albumin are the proteins made by the liver. Serum creatinine, a marker for the assessment of renal function is the most common indicator of glomerular filtration rate (GFR). A decrease in blood protein concentration during later stages of gestation was observed in sheep (Antunovic et al., 2002). It was also reported that plasma urea levels increased during week 10 of pregnancy, reaching a peak at parturition (El-Sherif and Assad, 2001), which in domestic ruminants was ascribed to the cortisol-stimulated catabolism of proteins in the body (Silanikove, 2000). The Creatinine clearance test could be used as a practical method for GFR assessment in the dromedary camel in field conditions (Kamili et al., 2013). The present study was initiated with the aim to investigate and compare some biochemical blood parameters in healthy male, female, and pregnant Bactrian camels.

Materials and Methods

This study was conducted on female and male Iranian two-humped camels (*Camelus bactrianus*) in April 2013. The camels were reared at the Bactrian camels research center, Jahadabad, (90 km far from the city of Meshkinshahr; 47° 43' 39.71" North latitude, 38° 26' 22.2" East longitude, and 1320 m above sea level) in Ardabil province, Northwest Iran. The camel herd composition includes a dominant male and 26 females at different ages. The camels were kept in an enclosed area with about 1000 m² of open space and pasture about 20 km². Twenty adult camels, aged between four and thirteen years old

were chosen for this study (Table 1). The pregnant camels were selected in consultation with cameleer who had recorded their mating history. Six non-pregnant camels, five in the last three months of pregnancy and nine male camels were selected for this experiment. All the animals were clinically healthy and free from internal and external parasites. The blood sample was collected into 10-ml vacuum tube and was chilled immediately after sampling and transported to the laboratory within 1 h after collection. Serum was harvested after centrifugation at 3000 rpm for 15 minutes, frozen, and stored at -21°C until analysis. Thyroid stimulating hormone (TSH), total tri-iodothyronine (tT3), and total thyroxine (tT4) concentrations were determined by radioimmunoassay method using commercial kits (Immuno-tech Company, Radiove, Prague, Czech Republic). The biochemical parameters were measured using a standard autoanalyser (Hitachi717, Boehringer, Mannheim, Germany). The level of total serum protein by Biuret reaction (Gornall et al., 1949), albumin by Bromocresol green dye binding method (McGinlay and Payne, 1988), and serum globulin was estimated by subtracting albumin from the total protein. Glucose and urea levels were measured with a clinical chemistry analyzer (Gilford Impact 400E, Gilford Systems, OH). The concentrations of calcium (Baginski et al., 1973) and inorganic phosphorus (Daly and Ertingshausen, 1972) were determined by an automated biochemical analyzer (Biotechnica, Targa 3000, Rome, Italy) using commercial kits (Parsazmoon, Tehran, Iran). The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by the colorimetric method of Reitman and Frankel (Mansour et al., 1982), and gamma glutamyltransferase (GGT) was measured by SZASZ method (Szasz, 1976). Serum enzyme activities were measured according to the specific reaction of each enzyme by using basic standard techniques. The serum was analyzed for cholesterol, HDL-cholesterol, and LDL-cholesterol by a modified Abell-Kendall/Levey-Brodie (AK) method (Burtis and Ashward, 1999), triglyceride by enzymatic method (Hinscha et al., 1980), and uric acid by phosphotungstic acid method (Elin et al., 1982). VLDL cholesterol was estimated as one-fifth of the concentration of triglycerides (Friedewald et al.,

1972). Total lipid (TL) was measured using Raylander et al.'s approach (2006), the overall regression equation ($\text{TL} = 0.9 + 1.3 * [\text{Cholesterol} + \text{Triglycerides}]$).

All results were expressed in SI unit (Burtis and Ashward, 1999). The data were analyzed by descriptive statistics and one way ANOVA followed by post hoc multiple comparisons of means using LSD tests with SPSS 16/PC software (Norusis, 1993). All values were expressed as mean and standard error (SEM) and ($p < 0.05$ and $p < 0.01$) were seen as statistically significant.

Results

The level of serum thyroid hormones (T3, T4, fT3 and fT4) and TSH are shown in Table 2. Table 3 shows the overall means \pm standard error (S.E.M) of the serum lipid profiles (Total cholesterol, triglyceride) and lipoproteins (HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol). Some of serum biochemical parameters and liver enzymes (AST, ALP, ALT and GGT) in pregnant, non-pregnant, and male Bactrian camels are listed in Table 4. The levels of T4, HDL-Cholesterol, ALP, and glucose in the sera of pregnant camels were significantly lower than those of the male and non-pregnant camels ($p < 0.01$ and 0.05 respectively). The serum levels of other parameters were not significantly different among the groups. In pregnant camels, the median serum thyroid hormones (T3, T4, fT3 and TSH) were lower than those of non-pregnant and male camels; nevertheless, the difference was not statistically significant. The average serum fT4 was significantly lower in pregnant camels than that of non-pregnant and males. There was no significant difference in serum lipid profiles and lipoproteins between non-pregnant, pregnant, and male camels; however, the value was lower in pregnant ones than that of non-pregnant and male ones. Serum creatinine and GGT were compared between these three groups, and results showed the values were higher in pregnant camels in comparison with non-pregnant and male camels; nonetheless, the differences were not statistically significant. We observed a decrease in serum AST level in pregnant camels compared to non-pregnant and male Bactrian camels.

Table 1. A summary of the age distribution of evaluating Bacterian camels.

| Camel/year | Number | Range | Minimum | Maximum | Median | Mean \pm Std. Error |
|--------------|--------|-------|---------|---------|--------|-----------------------|
| Pregnant | 5 | 3.00 | 10.00 | 13.00 | 13 | 11.80 \pm 0.73 |
| Non-pregnant | 6 | 1.00 | 4.00 | 5.00 | 5 | 4.66 \pm 0.21 |
| Male | 9 | 10.00 | 2.00 | 12.00 | 4 | 4.22 \pm 1.01 |

Discussions

The levels of fT4 in the sera of pregnant camels were significantly lower than those of the male and non-pregnant camels ($p < 0.01$) as well as for T3, T4, fT3, and TSH, although not significant (Table 2). Reduction in total circulating thyroid hormones during pregnancy could be due to the increase in turnover rate or the decrease in hormone secretion from the thyroid gland. During pregnancy, hepatocytes increase their production of thyroidbinding globulin (TBG). High TBG and high estradiol concentration during pregnancy induced a reduction in free circulating hormones such as fT4 (Utiger, 1987). Thyroid failure is more common in females and epidemiological rate of prevalence rises with age. The serum TSH assay is an accurate test for detecting out-of-range circulating levels of thyroid hormones for either of hypothyroidism and hyperthyroidism (Nouh et al., 2008). Estrogens can alter the secretion rate and dynamics of thyroid hormones. It seems that fluctuations in thyroid activity may be due to varying concentrations of estrogens and progesterone during pregnancy. Progesterone can decrease TBG, which increases free thyroid hormones. Estrogen increases TBG levels, which can inactivate thyroid hormone. These processes slow the body metabolism and allow the storage of fat and energy for the fetus (Agarwal et al., 1989). However, our findings are in agreement with some other investigators who found that free hormone levels remain unchanged or decrease in pregnancy (Rahman et al., 2007). Nazifi et al. (2003) and Manalu et al. (1997) found that the concentrations of serum T4 and T3 and the dosage were higher in non-pregnant goats than those of the pregnant ones. Also, they showed that the concentration of T4 was higher in female goats compared with males. Comparison of the HDL-cholesterol concentrations revealed significant lower values in the females than the males. Significant lower HDL-cholesterol concentration was observed in the pregnant camels ($p < 0.01$). The liver is the major site of cholesterol

synthesis and acetate was used as a precursor in this process. Cholesterol biosynthesis begins with the conversion of 3 Acetyl CoA units into Mevalonate. During pregnancy, the synthesis of cholesterol fell markedly (Leoni, 1984). There was no significant difference in other serum lipid profiles and lipoproteins between non-pregnant, pregnant, and male camels. The findings of the current study about lipid profiles in Bactrian camels do not support our previous researches in dromedary camel species (Omid et al., 2014). However, the findings of the current study are in agreement with the findings of Nath et al. (2005) and Krajnicakova et al. (2003) in cows, goats, buffaloes, and camels. The lower lipoproteins and lipid profiles level of these species near the parturition could be attributed to the increased utilization for steroid synthesis around parturition (Stocki, 1975). The quantity of creatinine depends on dietary intake, rate of synthesis of creatinine, and muscle mass. Serum creatinine and urea were compared between these three groups and results showed the values were higher in pregnant camels in comparison with non-pregnant and male ones; however, the differences were not statistically significant. Increase of creatinine concentration in pregnant animals could have been a consequence of the higher protein demands in late pregnancy which could have led to a reduction in the ability of kidneys to eliminate excess serotonin from plasma. Accordingly, Poljicak et al. (2009) found that the measured transaminase activity was higher in pregnant red and fallow deer females than non-pregnant ones. The higher activity of transaminases might indicate impairment in muscle and liver cells due to rapid gluconeogenesis associated with pregnancy. Beitz (2004) stated that the effect of adrenal corticoids on mobilization of amino acids from body proteins during pregnancy is associated with an increased rate of hepatic deamination. In the present research, there was no significant difference in serum total protein, albumin, and urea between non-pregnant, pregnant, and male camels; however, the values were lower in

Table 2. Mean \pm standard error of mean of serum thyroid hormones and TSH in pregnant, non-pregnant and male camels (n=20). T3 - triiodothyronine, T4 - thyroxin, fT3- free triiodothyronin, fT4- free thyroxin, TSH- thyroid stimulation hormone. (**) Significant difference in $p \leq 0.01$. NS Non significant difference. (a) In each row, indicates significant differences with two others.

| Variable | Unit | Bactrian camel | | | Significant |
|----------|----------|--------------------|--------------------|--------------------|-------------|
| | | Pregnant (n=5) | Non-pregnant (n=6) | Male (n=9) | |
| T3 | (nmol/L) | 114.56 \pm 17.87 | 149.56 \pm 14.94 | 121.46 \pm 15.69 | NS |
| T4 | (nmol/L) | 4.43 \pm 0.99 | 6.58 \pm 0.79 | 6.79 \pm 0.51 | NS |
| TSH | (mIU/L) | 0.01 \pm 0.001 | 0.005 \pm 0.001 | 0.01 \pm 0.001 | NS |
| fT3 | (pmol/L) | 1.86 \pm 0.18 | 2.55 \pm 0.31 | 2.58 \pm 0.29 | NS |
| fT4 | (pmol/L) | 0.64 \pm 0.11a | 0.97 \pm 0.06 | 1.06 \pm 0.06 | ** |

Table 3. Mean \pm standard error of mean of serum lipid profiles and lipoproteins in pregnant, non-pregnant and male camels (n=20). LDL- low-density lipoprotein, HDL- high-density lipoprotein, VLDL- very low-density lipoprotein, TL- total lipid. (**) Significant difference in $p \leq 0.01$. (NS) Non significant difference. (a) In each row, indicates significant differences with two others.

| Variable | Unit | Bactrian camel | | | Significant |
|-------------------|----------|------------------|--------------------|------------------|-------------|
| | | Pregnant (n=5) | Non-pregnant (n=6) | Male (n=9) | |
| Total Cholesterol | (mmol/L) | 27.60 \pm 3.70 | 32.16 \pm 2.95 | 36.11 \pm 2.80 | NS |
| Triglycerid | (mmol/L) | 23.80 \pm 2.65 | 36.66 \pm 6.60 | 34.00 \pm 2.71 | NS |
| HDL- Cholesterol | (mmol/L) | 7.00 \pm 0.89a | 15.33 \pm 1.80 | 18.11 \pm 1.08 | ** |
| LDL- Cholesterol | (mmol/L) | 15.8 \pm 4.31 | 9.83 \pm 1.45 | 11.22 \pm 1.10 | NS |
| VLDL- Cholesterol | (mmol/L) | 7.33 \pm 0.96 | 7.33 \pm 0.88 | 6.80 \pm 0.72 | NS |
| Total Lipid | (g/L) | 67.8 \pm 10.09 | 90.38 \pm 9.21 | 91.93 \pm 7.52 | NS |

Table 4. Mean \pm standard error of mean of some of serum biochemical parameters and liver enzymes in pregnant, non-pregnant and male camels (n=20). AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, Gama glutamine transferase. (*) Significant difference in $p \leq 0.05$. (**) Significant difference in $p \leq 0.01$. (NS) Non significant difference. (a) In each row, indicates significant differences with two others.

| Variable | Unit | Bactrian camel | | | Significant |
|---------------|--------|---------------------|--------------------|--------------------|-------------|
| | | Pregnant (n=5) | Non-pregnant (n=6) | Male (n=9) | |
| Glucose | mmol/L | 68.60 \pm 16.01a | 106.83 \pm 3.02 | 104.77 \pm 5.39 | * |
| Calcium | mmol/L | 10.78 \pm 0.35 | 9.55 \pm 0.199 | 10.1 \pm 0.35 | NS |
| Phosphorus | mmol/L | 4.58 \pm 0.61 | 7.08 \pm 0.66 | 7.18 \pm 0.78 | NS |
| Albumin | g/L | 2.86 \pm 0.25 | 2.98 \pm 0.22 | 3.03 \pm 0.15 | NS |
| Total protein | g/L | 5.74 \pm 0.31 | 5.76 \pm 0.37 | 5.84 \pm 0.24 | NS |
| Urea | mmol/L | 60.80 \pm 7.52 | 65.17 \pm 7.09 | 63.78 \pm 5.88 | NS |
| Uric acid | mmol/L | 0.2 \pm 0.04 | 0.22 \pm 0.06 | 0.18 \pm 0.5 | NS |
| Creatinine | mmol/L | 1.62 \pm 0.17 | 1.38 \pm .07 | 1.41 \pm 0.09 | NS |
| AST | IU/L | 89.4 \pm 9.41 | 151.83 \pm 24.53 | 184.11 \pm 35.16 | NS |
| ALT | IU/L | 12.8 \pm 1.49 | 14.5 \pm 1.98 | 15.11 \pm 1.15 | NS |
| ALP | IU/L | 109.40 \pm 10.01a | 345.66 \pm 68.36 | 381.77 \pm 51.92 | ** |
| GGT | IU/L | 21.8 \pm 1.49 | 21.5 \pm 1.12 | 20.33 \pm 0.88 | NS |

pregnant camels than those of non-pregnant and male ones. With insufficient water supplies, there is an evidence of an increase in blood total protein, albumin, and urea concentration along with the pregnancy progress (El-Sherif and Assad, 2001; Poljicak, 2009). Rodriguez et al. (1996) found that glomerular filtration and urea clearance were significantly reduced during late pregnancy. In our study, the levels of glucose in the sera of pregnant

camels were significantly lower than those of the male and non-pregnant camels. Our findings were in agreement with the findings of some researchers, e.g. Khan and Ludri (2002) and Saeed et al. (2009): They found significantly lower concentrations of glucose in pregnant goats than in non-pregnant ones with a tendency to decline in the group of the pregnant animals towards the end of gestation. The low level of glucose in pregnant camels may be due to developing

fetus and mobilization of glucose from mother for providing the adequate energy of the fetus. Normal blood contains alkaline phosphatase (ALP) enzyme which catalyses the liberation of inorganic phosphates from phosphate esters (Bodansky, 1932). In this study, the level of ALP in male and non-pregnant camels was significantly higher than that of the pregnant camels. The level in the healthy animal is influenced markedly by age and to some extent by diet activity. The plasma ALP levels may increase during the diseases related to bone and liver (Shinowara, 1942). In cattle, sheep, and camels the effect of age on ALP was noticed by Vertor and Swaton (1969), where the serum ALP activity was considerably higher in young animals. A high level of ALP was reported by Elias and Yagil (1984) in the newborn calves. In Indian camels, ALP activities in the serum of male animals were significantly higher than those of the female animals (Kataria and Bhatia, 1991). Animals younger than three years of age had higher ALP activity than adult males. Progressive decline in ALP activity with the advancement of age in camels was also observed (NRCC, 1990). In this study, pregnant female camels were significantly older than the group of non-pregnant and male camels (Table 1). The ALP activity obtained from pregnant and non-pregnant camel was higher than the values reported by other researchers (Saeed et al., 2009; Khadjeh, 2002). There is not any relationship between the serum calcium or phosphorus levels and the variations observed in the ALP in pregnant and other camels (Table 4). It should be noted that all determinations were made with apparently healthy camels. No explanation other than an insufficient number of samples is readily available for the high and wide variation in serum ALP levels. The Major limitation of this study is that the number of Bactrian camels was relatively small in Iran (less than 200). Bactrian camel's research center in Jahadabad of Meshkinshahr is the only center of rearing two-humped camels. Our results may be influenced by this limitation. Future studies on the current topic are recommended.

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سیمای متابولیک شترهای دو کوهانه (*Camelus bactrianus*) آبستن، غیرآبستن و نر ایران

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چکیده

زمینه مطالعه: دانش در خصوص ترکیبات خون برای ارزیابی وضعیت فیزیولوژیکی و سلامت حیوانات مهم است. تنها تعداد معدودی شتر دوکوهانه (*Camelus bactrianus*) در استان اردبیل در شمال غربی ایران پرورش داده می‌شوند. **هدف:** مطالعه حاضر به منظور بررسی اثر جنسیت و آبستنی بر هورمونهای تیروئیدی، سیمای لیپیدی و لیپوپروتئینی و فاکتورهای بیوشیمیایی منتخب شترهای دوکوهانه سالم ایرانی انجام شد. **روش کار:** گله مورد بررسی شامل بیست شتر دوکوهانه سالم از نظر بالینی، بین سنین چهار و سیزده سال بود. شش نفر از آنها آبستن نبودند؛ پنج نفر در اواخر آبستنی و نه شتر نر بودند. نمونه خون (۱۰mL) از ورید وداج شترها در بهار ۲۰۱۳ در طول دو روز متوالی جمع‌آوری شد. تمام نمونه‌ها در دور ۳۰۰۰ به مدت ۱۵ دقیقه سانتریفوژ شدند و سرم در فریزر ۲۱°C- تا زمان آزمایش نگهداری شد. آزمون‌های عملکردی تیروئید از طریق اندازه‌گیری سطوح سرمی هورمون محرک تیروئید (TSH)، تیروکسین آزاد (fT4) و تام (T4) و تری‌یدوتیرونین آزاد (fT3) و تام (T3) توسط کیت رادیوایمونواسی تجاری در دسترس، انجام شد. پارامترهای بیوشیمیایی با استفاده از اتوانالایزر استاندارد (Hitachi 717, Boehringer. Mannheim, Germany) اندازه‌گیری شد. **نتایج:** سطوح fT4 و HDL کلسترول، ALP، و گلوکز در سرم شترهای آبستن به طور قابل توجهی پایین تر از شترهای نر و غیرآبستن بود. سطوح سرمی پارامترهای دیگر بین گروه‌ها متفاوت نبود. **نتیجه‌گیری نهایی:** مطالعه حاضر نشان داد که آبستنی سنگین تاثیر عمیقی بر برخی پارامترهای بیوشیمیایی سرم در شترهای دوکوهانه دارد.

واژه‌های کلیدی: شتر دوکوهانه، انتهای آبستنی، شتر نر

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