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The Evaluation of Reproduction in Bactrian Camels (*Camelus bactrianus*) and the Possibilities of Using Non-invasive Methods for Detection of Heat and Pregnancy

PhD Thesis

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STATEMENT

I declare that I conducted this dissertation thesis, entitled "The Evaluation of Reproduction in Bactrian Camels (*Camelus bactrianus*) and the Possibilities of Using Non-invasive Methods for Detection of Heat and Pregnancy" alone and I used the literature mentioned in the references. All photographs in this document were taken by myself.

In Prague, 12.08.2015 ....

Tamara Fedorova

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ABSTRACT

Thesis title: The Evaluation of Reproduction in Bactrian Camels (*Camelus bactrianus*) and the Possibilities of Using Non-invasive Methods for Detection of Heat and Pregnancy

Camels are important husbandry animals which are also often bred in zoological gardens. Unfortunately, camels in European zoos are not usually trained and pregnancy diagnosis in a half-tamed camel is very difficult. Moreover, information of the maternal behaviour of camels is limited. This thesis reviewed current knowledge on camel husbandry, reproduction and behaviour and aimed to 1) examine non-invasive methods of heat and pregnancy diagnosis from urine and saliva in camels kept in zoological gardens; 2) explore their maternal and suckling behaviour; 3) describe experiences with artificial rearing of camel calves.

The research into non-invasive pregnancy diagnosis was carried out from 2010 to 2012. Urine from 14 camel females kept in four European zoological gardens was collected and tested using two chemical tests – the Cuboni reaction and barium chloride test. The Cuboni reaction was significantly ($p < 0.01$) affected by the pregnancy status of female camels, and its accuracy increased significantly ($p < 0.05$) in the period leading up to parturition. The barium chloride test did not provide reliable results. Next, the saliva of five adult female camels was sampled for more than one year and concentrations of progesterone (P4) and oestradiol (E2) were measured. The concentrations of P4 ($n = 312$) and E2 ($n = 310$) were both significantly ($p < 0.0001$) affected by the pregnancy status of the animals. Maternal and suckling behaviour was observed from 2003 to 2009 in six zoological gardens, and the presented study includes partial data from this period. Allosuckling (i.e. when a female nurses a non-filial offspring) was described for the first time in camels and it represented 8.58% of all suckling bouts. The non-filial calves suckled more often in the lateral position and preferably joined the filial calf when suckling, so the results support the 'milk theft' hypothesis (stealing of milk) as a main cause of this behaviour. Finally, calf rearing in the Prague zoological garden was summarised and two camel calves were successfully artificially reared.

This PhD thesis concluded that 1) the Cuboni reaction with urine and salivary P4 and E2 measurements are suitable methods for pregnancy diagnosis in half-tamed female camels; 2) allosuckling is relatively common in captive Bactrian camels; 3) the artificial rearing of camel calves with a calf milk replacer can be successful.

Keywords: Allosuckling, artificial calf rearing, Cuboni reaction in urine, maternal behaviour, salivary oestradiol and progesterone.

ABSTRAKT

Název práce: Zhodnocení reprodukce velbloudů dvouhrbých (*Camelus bactrianus*) a možností využití neinvazivních metod detekce říje a březosti

Velbloudi jsou nejen významnými hospodářskými zvířaty. Bývají také poměrně často chováni v zoologických zahradách, kde jsou ale zřídkakdy trénováni. Diagnostika březosti je však u částečně ochočených velbloudů velmi obtížná. Navíc informací o mateřském chování velbloudů je k dispozici velmi málo. Tato práce shrnuje dosavadní poznatky o chovu velbloudů, jejich reprodukci a etologii a měla za cíl 1) otestovat neinvazivní metody zjišťování říje a březosti z moči a slin velbloudů chovaných v zoologických zahradách; 2) prostudovat jejich mateřské chování a kojení; 3) popsat zkušenosti s umělým odchovem velbloudích mláďat.

Výzkum neinvazivní diagnostiky březosti se konal v letech 2010 až 2012. Moč byla odbírána od čtrnácti velbloudích samic ze čtyř zoologických zahrad v rámci střední Evropy a diagnostika březosti probíhala pomocí Cuboniho reakce a testu chloridem barnatým. Výsledky Cuboniho reakce byly významně ($p < 0.01$) ovlivněny fází březosti samic a spolehlivost toho testu se zvyšovala ($p < 0.05$) s časem blížícího se porodu. Test chloridem barnatým nepřinesl spolehlivé výsledky. Dále byly u pěti velbloudic po dobu více než jednoho roku pravidelně odebírány vzorky slin, ze kterých byly stanoveny hladiny progesteronu ($n = 312$) a estradiolu ($n = 310$). Koncentrace obou hormonů byly významně ($p < 0.0001$) ovlivněny fází březosti samic. Mateřské chování velbloudích samic a chování jejich mláďat bylo dlouhodobě sledováno od roku 2003 do roku 2009 v šesti zoologických zahradách, nicméně v této práci byla zahrnuta pouze část ze získaných dat. Naše studie jako první prokázala výskyt tzv. alokojení (tedy jev, kdy samice kojí i jiná než vlastní mláďata). Toto chování, s výskytem 8.58% ze všech kojení, se ukázalo jako poměrně běžné. Cizí mláďata sála nejčastěji v pozici co nejdále od hlavy kojící samice a preferovala sání spolu s vlastním mládětem, proto byla hypotéza krádeže mléka vyhodnocena jako nejpravděpodobnější příčina alokojení. V neposlední řadě práce shrnuje odchovy mláďat velbloudů v pražské zoo a popisuje umělý odchov dvou velbloudích mláďat.

Tato práce dospěla k následujícím závěrům: 1) Cuboniho reakce moči a stanovení progesteronu a estradiolu ze slin jsou použitelné metody pro zjišťování březosti velbloudů; 2) alokojení je poměrně běžným jevem u dvouhrbých velbloudů v lidské péči; 3) umělý odchov velbloudích mláďat pomocí mléčné krmné směsi pro telata může být úspěšný.

Klíčová slova: Alokojení, umělý odchov, Cuboniho reakce moči, mateřské chování, estradiol and progesteron stanovený ze slin.

CONTENT

1. INTRODUCTION

Camels are important husbandry animals in many parts of the world. The total camel population was estimated to be almost 27 million in 2013, and the number of camels in the world almost doubled over the last 50 years (FAO, 2015). Camels are also often bred in zoological gardens; according to the Zoological Information Management System (ZIMS), 190 European zoological gardens or similar institutions keep camels (ISIS, 2014). About 2,500 camels are kept in the USA, mainly by private keepers (Baum, 2011).

The management of reproduction and pregnancy diagnosis are very important and decisive for the successful breeding of every species in captivity (Ramsay et al., 1994; Kleiman, 2010). But animals in zoos are not truly domesticated, so every handling or medical examination can be stressful for animals, often requiring their physical or chemical restraint, and can be dangerous for the animals and also personnel (Christman, 2010). Additionally it is often impossible to recognize the signs of oestrus in female camels (Musa et al., 2004) and the oestrus behaviour of female camels is not always associated with the presence of a dominant follicle (Skidmore et al., 1996a; Vyas and Sahani, 2000). Common methods of pregnancy confirmation in tamed camels such as rectal palpation, vaginal examination, transrectal or endovaginal ultrasonography (Banerjee et al., 1981; Skidmore et al., 2000; Vyas and Sahani, 2000; Khatir and Anouassi, 2006) or blood progesterone analysis (Bakheit et al., 2008) are not usually applicable in half-tamed camels kept in European zoological gardens and pregnancy can often only be estimated according to morphological changes in the females in the final months of gestation (e.g. increasing size of abdomen or udder).

Camels are usually bred as multipurpose husbandry animals; mostly for work, meat or milk production, for hair and hides (Mukasa-Mugerwa, 1981; Asmare, 2000) and so research focuses mainly on these areas, together with camel reproduction and nutrition. Only a few studies focus on the behaviour of camels, despite the fact that knowledge of the animals' behaviour is closely connected to their management, reproduction and welfare. Moreover, the behaviour of animals in captivity often differs from their natural behaviour in the wild (Thompson, 2010).

For these reasons, research into non-invasive heat and pregnancy diagnosis and maternal behaviour in captive camels is needed. The main aim of this PhD thesis was to test several methods of pregnancy or heat diagnosis in camels and investigate the maternal behaviour of camels bred in zoological gardens (see Chapter 3. for the specific objectives of the thesis).

2. LITERATURE REVIEW

2.1. Life History and Facts about Camels

Camels, together with llamas, evolved from a common ancestor about 40 million years ago in North America. While the ancestors of llamas migrated to the south, ancient camels crossed the Bering Isthmus (land bridge) to Asia six million years ago (Prothero and Schoch, 2002; Rybczynski et al., 2013). Camelid evolution is well described by Prothero and Schoch (2002). Camels were domesticated probably 5,000 years ago for riding and as pack animals (Faye, 2012).

Traditionally two camel species, dromedary (*Camelus dromedarius,* Linnaeus 1758) and Bactrian camels (*Camelus bactrianus,* Linnaeus 1758), were classified in the order Artiodactyla (even-toed ungulates), suborder Tylopoda, family Camelidae (ITIS, 2012). The order Artiodactyla and order Cetacea are now often grouped together in the single order Cetartiodatyla (Gatesy et al., 2002; Price et al., 2005; Groves and Grubb, 2011). Next, the third camel species – the critically endangered Wild Bactrian camel (*Camelus ferus*) is now recognized by some authors, and a breeding program for this unique species is underway in Mongolia (Hare, 2008; Ji et al., 2009; Groves and Grubb, 2011; Jirimutu et al., 2012).

Currently, more than 80% of the camel population lives in Africa (FAO, 2015), where dromedary camels are found (Rischkowsky and Pilling, 2007). Dromedaries are also known as Arabian or one-humped camels and they are bred mainly in Northern Africa, the Middle East, India and Pakistan (Rischkowsky and Pilling, 2007), but they can also be found in southern Africa, the USA, Australia, South America and the Caribbean (Wilson, 1984; Franklin, 2011). The biggest herds are found in Somalia, former Sudan, Kenya, Niger and Mauretania (FAO, 2015). Besides FAOSTAT data, more than one million camels live in Australia. The feral population of dromedary camels poses a big threat for the local ecosystem as well as for native animals, and causes serious socio-economic problems (Grigg et al., 1995; Spencer et al., 2012). But this population also provides benefits for local farmers, who use camels for racing or meat production. Moreover, feral camels offer a unique opportunity for behavioural studies (Edwards et al., 2008).

The Bactrian camel, also called the two-humped camel, is kept as a husbandry animal mainly in Mongolia, China and Kazakhstan (Rischkowsky and Pilling, 2007). A small population of Wild Bactrian camels still survives in Mongolia and China, numbering less than 1,000 animals (Hare, 2008).

According to FAOSTAT data and estimations, world camel population is growing overall (FAO, 2015) but for example Franklin (2011) stated that the population of dromedaries decreased during the second half of the 20^{th} century. Camel population statistics are usually crude estimates, and all evidence is hard to obtain due to nomadic herds migrating across countries (Bourzat and Wilson, 1987). I can conclude that the population trend in last 50 years differs across regions. The camel population is decreasing in Asia, mainly in India, China and Mongolia, while it is increasing in Africa, the Arabian Peninsula and Pakistan. But differences across individual countries also exists (FAO, 2015). As a result of the huge camel population decline in Asia, the number of Bactrian camels decreased to 600,000–700,000 (Köhler-Rollefson, 2005; FAO, 2015).

Traditionally, there are two recognised types of camel use: for riding and as pack animals (Khanvilkar et al., 2009a). Older literature also says that camels had no breeds and only recognised some local forms (Vohradský, 1999). Recently, a number of authors have written about different camel breeds selected for specialized uses (Köhler-Rollefson, 1993; Wardeh, 2004) and Wardeh (2004) newly classified camels into the meat type, dairy type, dual purpose (where most camels for riding and as pack animals are included) and racing camels. Furthermore, camels are sometimes kept as pet animals or for sports (Yilmaz et al., 2011).

Camels are large husbandry animals. Dromedaries weigh on average 450–590 kg (Khanvilkar et al., 2009a) and some breeds reach a height of more than 200 cm at the withers (Wardeh, 2004). Bactrian camels with an average weight between 500 and 800 kg are more massive; some males can weigh more than one ton (Baimukanov, 1989). The camel's head is relatively big, with large eyes and small ears (Wilson, 1984). Long eyelashes and lockable nostrils protect the animals from sand (Sambraus, 1994). The foot is comprised of two digits, the third and fourth; the other digits have disappeared. The foot is protected by soft pads of 7-mm-thick modified skin. Other pads can be found on the chest, elbows and stifles and smaller pads are on the knees and hocks (Wilson, 1984; Franklin, 2011). The pads enable camels to lie on hard and hot surfaces (Lensch, 1999; Franklin, 2011). The body is covered with short fine hair; longer hair is usually located on the hump and shoulders (Wilson, 1984). Hair colour varies in different shades of brown, from light to very dark. Also white, black and even multi-coloured animals can be found, though they are rare (Raziq and Younas; Abdussamad et al., 2011; Franklin, 2011).

The camel's sweat glands are dispersed all over its body. Poll glands which developed form sweat glands are active mainly in males during rut or in animals under stress (Tingari et al., 1984; Wilson, 1984; Vernerová, 1990). The hump is composed of fibrous fatty tissue. The dromedary has a relatively elastic hump, but the humps of Bactrian camels are largely inflexible and often flop over on one side (Wilson, 1984; Lensch, 1999). The size of the hump is not only affected by the body's condition but genetic factors could play a role (Faye et al., 2001). Camels are ruminants, but they have a different anatomy of the digestive tract from *Ruminantia*. Camels lack one fore-gut and so digest via only three compartments (Miller and Fowler, 2014). However according to some studies, the digestive system of camels is more effective than that of cattle (Lechner-Doll et al., 1995).

The physiology of camels is unique, and it helps them to survive in extreme environments. Firstly, camels can very effectively save water. Their body temperature varies according to temperature of the environment, efficient kidneys produce very concentrated urine and camels only start to sweat when their body temperature reaches 40°C (Schmidt-Nielsen et al., 1956b; Schmidt-Nielsen et al., 1967; Wilson, 1984). Next, camels can survive several weeks without water, and they can lose over 25% of their body weight by dehydration in extreme conditions (Schmidt-Nielsen et al., 1956a; Silanikove, 1994). Moreover, the fat in humps is a useful source of energy for a period of starvation (Bengoumi et al., 2005).

Camels are multipurpose animals. Dairy breeds reach an average yield of over 3,000 kg (see Chapter 2.2.9. for details) (Aujla et al., 1998; Wardeh, 2004). Camel meat is also very popular in some areas. The major producers of camel meat over the last decade were Sudan, Saudi Arabia, Somali, Egypt and Kenya (FAO, 2015). The taste is similar to beef with a dressing percentage of about 55–65%. Working camels are usually used for the transport of humans and loads. Their carrying capacity is about 25–40% of the camel's body weight. Camels are also utilised for draught, ploughing, pulling water from wells and in milling. Camel hair is used for the manufacture of blankets, ropes and mats (Khanvilkar et al., 2009a). The hair of the Bactrian camel is longer than in the dromedary as a result of its adaptation to extremely cold winters in the Gobi desert (Franklin, 2011). Bactrian camels naturally shed in the spring. The hair yield is one or two kilograms from dromedary (Meredov, 1989; Chand et al., 2011) and up to 16 kg from Bactrian camel (Baimukanov, 1989). Camel dung serves as a fuel, and bones as a fertiliser (Khanvilkar et al., 2009a). Camels are also used for sport similar to horses. Camel races (Wardeh, 2004) and even polo (Turtureanu, 2010) are common in some areas. Moreover camel dancing is popular in Pakistan (Ali et al., 2009) and camel wrestling in Turkey (Çalışkan, 2010; Yilmaz et al., 2011).

2.1.1. Behaviour and Ecology

Camels are very social animals, but few studies about their behaviour have been published, and most of them were conducted on wild populations (Franklin, 2011). Nevertheless, the behaviour of Wild Bactrian camels is almost unknown due to their timidity (McCarthy, 2000) and the behaviour of feral camels has rarely been studied. As a result, our knowledge of the natural behaviour of camels is very limited.

The movement and distribution of Wild Bactrian camels mainly depends on water and food sources. The rut occurs between November and February. In this period, the dominant bull collects a harem of about ten to twenty cows. Most births take place in March and April. Females have one calf every second year. The female separates from the herd before parturition and stays alone with her calf for about two weeks (Tulgat and Schaller, 1992; Schaller, 1998). The average observed herd size was from 10 to 26 animals (Reading et al., 1999; McCarthy, 2000). In Wild Bactrian camels, it was almost never possible to get closer to determine more concrete information about herd structure, sex ratio and similar data (McCarthy, 2000).

The social organisation of feral camels in Australia and their behaviour was observed for many years by Dörges and Heucke (1995). Camels' home range varies according to season and annual rainfall from 450 to 5,000 km^2. Camels are non-territorial and females with calves, sub-adult males and heifers form herds which are led by an experienced old female. Adult bulls live alone or in bachelor groups and only approach herds during breeding season. The adult dominant bull forms his harem and sub-adult bulls are bundled away from the herd. Rutting bulls can also be aggressive towards the new-born calves and infanticide was observed. This could be a probable explanation for why females leave the herd before parturition (Dörges et al., 1992; Dörges and Heucke, 1995; SCARM, 1997; Ellard and Seidel, 2000; Edwards, 2008).

The behaviour of domestic camels is affected by their management, but some authors found similar behavioural patterns for camels in captivity to camels in the wild. Herd size varies from 4 to 33 animals for semi-wild, extensively managed camels (Franklin, 2011). Herds are also usually led by older females (Gauthier-Pilters, 1971; Schulte and Klingel, 1991). The bull is the dominant animal mainly during rutting-related activities, but in other situations males commonly command no respect from females (Schulte and Klingel, 1991; Haberová, 2009). Camel bulls chase young males when they approach too close to the herd. No hierarchy among females was observed (Schulte and Klingel, 1991).

Browsing or grazing are camels' predominant daily activities (Kassilly, 2002; Dereje and Uden, 2005; Mengli et al., 2006; Chimsa et al., 2013). Adult animals spent about 65% of daytime feeding (Dereje and Uden, 2005) and calves even more, up to 82% of their time (Chimsa et al., 2013). The camels' diet differs throughout the year in nature, and consists of a lot of species of shrubs and forbs (Gauthier-Pilters, 1971; Mengli et al., 2006). Camels often eat thorny and woody plants (Gauthier-Pilters, 1971).

Camels usually rest in small groups in a sternal recumbency position with their faces towards the sun to minimise the amount of the sun's rays falling on them (Gauthier-Pilters, 1971; Sedláček, 2014). Herd members often copy each other's behaviour; when one animal starts to do some activity, the rest of herd will usually join in. Camels can be easily scared by unusual objects (Gauthier-Pilters, 1971).

Oral and locomotor stereotypy was observed in a captive camel kept in isolation (Padalino et al., 2014). Sometimes, wool plucking and eating was observed in camel calves (Puschmann et al., 2013).

2.1.2. Herd Management

Camels are kept under traditional pastoral management in many parts of Asia (Jasra et al., 1999; Ishii and Samejima, 2006) and Africa (Farah et al., 2004; Kalla et al., 2008). The herds are either home-based or nomadic. The management of reproduction in camels bred in the traditional way is almost non-existent, and males mate with females freely (Farah et al., 2004; Kalla et al., 2008). The camels are sometimes combined with herds of cattle, sheep or goats (Abbas et al., 1992).

Animals often live on natural pasture with a low nutritive value; only salt and water are provided every few days (Farah et al., 2004; Bakheit et al., 2008). Camels are mainly sorted into males and females. All females are usually used in reproduction and the herder often uses the same bull for many years. This bull frequently originates from the same herd. This is probably one of the causes of the relatively high rates of calf mortality (Kohler-Rollefson et al., 1991; Farah et al., 2004), together with the fact that some owners believe that colostrum causes diarrhoea in calves, and they feed calves with mature milk (Farah and Fischer, 2004). Older calves are often not allowed to suckle milk during the day, and the udder of the female is protected by a cover to save milk for human use (Gauthier-Pilters, 1971).

Sometimes multiple bulls are bred together and the strongest bull mates with females (Kohler-Rollefson et al., 1991). While females are preferred for breeding purposes, young bulls are often culled (Elmi, 1989; Farah and Fischer, 2004) or reared, later castrated and used for work (Wilson, 1984; Abbas et al., 1992). In some countries, males are only kept together with females during the breeding season (Aboulela, 1994).

The number of farmers practising semi-intensive breeding techniques has been increasing in recent years. It provides camels with more comprehensive care, e.g. supplementary feed in addition to pasture, *ad libitum* watering and basic health care. Camels bred in a semi-intensive system have better production and also reproduction performances (Bakheit et al., 2008). One bull is usually bred together with 11 to 25 females (Aboulela, 1994; Tefera and Gebreah, 2001), but sometimes with up to 40 or 50 females (Kohler-Rollefson et al., 1991; Padalino et al., 2015).

Modern breeding systems mainly exist for racing camels and for highly productive dairy camel females. These breeding facilities are typical mainly for the Arabian Peninsula, but modern research centres focusing on camels and their performance can be found e.g. in India (Agarwal et al., 2003), the United Arab Emirates (Tinson et al., 2001) and Saudi Arabia (Gaili et al., 2000).

Camels are also kept as hobby animals in various parts of the world. Private keepers usually own one or two camels but bigger camel farms can be found for example in the USA (Baum, 2011), Australia (Edwards et al., 2008), Turkey (Yilmaz et al., 2011) and even in England (http://jacamels.co.uk) and the Czech Republic (www.velbloudi.cz). Camels at these farms are often also used for tourism.

In zoological gardens, camels are usually kept in smaller groups composed of one adult bull and usually up to 15 camel females (Puschmann et al., 2013). Calves can stay in the herd for up to two years, later the bull may start to attack young bulls or to mate with his daughters (Holečková and Dousek, 2006; Puschmann et al., 2013). The whole herd is usually kept together throughout the year and the female can give birth in the herd, however the separation of the female is sometimes beneficial (Puschmann et al., 2013). In the experience of the author of this PhD thesis, some females preferred to be separated before parturition, while others were more stressed in isolation. The aggression of herd members towards a calf is rare, but some bulls regularly attack new-born calves and must be separated from the herd. Camels can be combined with other animal species, but conflicts between the camel bull and the adult male of larger species, e.g. a bull of a yak (*Bos grunniens*. and *Bos mutusor*) or stallion of a kulan (*Equus hemionus kulan*) have been observed (Puschmann et al., 2013).

Camels can be easily manageable when they undergo basic training, but due to their size the keeper must always be careful. Aggression is usually increased in bulls during the rut. Camels defend themselves or attack rivals by kicking, biting and spitting saliva (Yagil and Etzion, 1980; Abu-Zidan et al., 2012; Puschmann et al., 2013; Miller and Fowler, 2014). Trained camels are docile (Gauthier-Pilters, 1971), however training which maintains the animals' welfare is time-consuming (SCARM, 1997; Osborne, 2011), and so camels are not usually trained in Czech zoological gardens.

2.2. Reproduction in Camels

2.2.1. Anatomy of Reproductive Organs

The female genital tract is similar to that of the mare (Yagil, 2006), nevertheless some traits are more comparable to cattle or swine. The size of the ovaries is mainly determined by the age and reproductive stage of the female, but the average dimensions in an anoestrous camel female are 2.6 × 2.2 × 0.9 cm (ElWishy, 1988). Oviducts are 14–30 cm long with distinct circular papillae. The uterus is bipartite and is T-shaped. The cervix is short (about 0.36 cm) in contrast to the vagina, which is very long (about 32 cm) (Srikandakumar et al., 2001). The left uterine horn is often bigger, well developed and more active than the right one (ElWishy, 1988; Al-Eknah et al., 2001; Srikandakumar et al., 2001). The placenta is diffuse and epitheliochorial (Abd-Elnaeim et al., 1999; Wooding et al., 2003). The female camel has a four-quartered udder which is covered with a thin black skin (Wilson, 1984).

The male's testicles are situated high up in the groin (Wilson, 1984; Merkt et al., 1990). The average length is between 9.3 and 9.5 cm (Djang et al., 1988). Their weight varies from 57 g in non-breeding season to 109 g in breeding season (Tingari et al., 1984). The penis is S-shaped and its average length is 52.5 cm (Djang et al., 1988). Male camels have prostate, bulbourethral glands, a urethra and an ampulla ductus (Ali et al., 1978). The opening of the urethra is directed backwards, and so the male camel urinates in this direction. Powerful protractor muscles erect the penis forwards prior to mating (Wilson, 1984; Merkt et al., 1990).

2.2.2. Male Reproductive Behaviour

Camel males reach sexual maturity at the age three or four years, but they mostly reproduce about two years later (Rahim, 1997; Al-Qarawi et al., 2001; Ali et al., 2009). The behaviour of a rutting bull is very specific and easily recognisable. The bull often grinds his teeth and slobbers large amounts of white foam. He urinates more frequently and spreads urine onto his body with his tail. The bull also rubs the poll gland against the hump (Figure 1) or other available surfaces on his body. A brown and pungent secretion is produced by this gland (Yagil and Etzion, 1980; Bhakat et al., 2005; Padalino et al., 2015). Dromedaries also blow through the Dulaa (the balloon-like organ of palatal flap) from the mouth. The typical sound of males in the rut is “bloo, bloo, bloo” (Yagil and Etzion, 1980; Yagil, 2006). The bulls are very interested in camel females, smell their urine and flehmen (Figure 2) (Yagil and Etzion, 1980; Padalino et al., 2015).

The male in rut very often becomes aggressive towards other males (Yagil, 2006; Padalino et al., 2015) and also towards keepers, and every manipulation is very difficult (Yagil and Etzion, 1980; Puschmann et al., 2013; Miller and Fowler, 2014). Males fight with each other with their necks (Gauthier-Pilters, 1971), trying to force their rivals onto the ground.

Figure 1: Male rubbing the hump.

Figure 2: Flehmening behaviour.

2.2.3. Seasonality

Camels are seasonal breeders (Abdel-Raouf et al., 1975; Boness et al., 1998; Al-Hazmi, 2000; Nowshari and Ali, 2005; Musa et al., 2006; Ali et al., 2008; Kalla et al., 2008; El-Harairy and Attia, 2010; El-Harairy et al., 2010). Breeding season depends on the area of distribution and on the climate, temperature, humidity, daylight length and rainfall (Bono et al., 1989; El-Harairy and Attia, 2010). The breeding seasons in different countries are summarized in Appendix 1. Calving season reflects the breeding season, only the length of gestation (ca 13 months; see Chapter 2.2.6.) needs to be added to the calculation (Aboulela, 1994).

2.2.4. Female Oestrous Cycle

The length of the cycle in camel females varied across the studies from 11 to 30 days (Elias et al., 1984; Homeida et al., 1988; Al-Eknah et al., 1993; Alfuraiji, 1999; Musa et al., 2006). Oestrus usually last from 5 to 8 days (Skidmore et al., 1996a; Alfuraiji, 1999) .

The oestrous cycle of a camel female is usually characterised by three phases which correspond with ovarian follicular development: the phase of growing follicles (proestrus), mature follicles (oestrus) and regression of follicles (diestrus) (Marai et al., 2009). Ovulation is induced by mating in camels (McKinnon et al., 1994; Zhao et al., 1994) so the oestrus cycle is not usually complete compared to other ungulates (Marai et al., 2009), because the corpus luteum is absent in non-mated females and so the luteal phase is missing (Elias et al., 1984).

A follicular wave begins when the dominant (the biggest) follicle starts to grow. The dominant follicle suppresses the growth and maturation of other follicles. The mature follicle phase (oestrus) starts when the dominant follicle stops its growth and ends when it loses its dominance over other follicles. After that, the dominant follicle regresses or develops into an oversized follicle (Skidmore et al., 1996a; Manjunatha et al., 2012). The presence of oversized follicles is common in camels; its occurrence was observed in 44% (Skidmore et al., 1996a) and even 73% of all follicular waves (Manjunatha et al., 2012). A dominant follicle reaches on average 2 cm (with a range of 1.5–2.5 cm) while an oversized follicle can reach up to 6 cm. A size between 0.9–1.9 cm indicates the optimal time for mating (Skidmore et al., 1996a). According to Skidmore et al. (1996a), no oversized follicles were found in females mated by vasectomized male, and it is possible that ovulation after mating, even if unsuccessful, is a natural part of the camel oestrus cycle.

A new follicular wave starts before the regressive phase of the previous wave ends by complete regression of the former dominant or oversized follicle (Skidmore et al., 1996a; Manjunatha et al., 2012). The length of a complete follicular wave lasted on average from 28 to 47 days in dromedary camels (Skidmore et al., 1996a; Alfuraiji, 1999; Manjunatha et al., 2012) and 44 days in Bactrian camels (Nikjou et al., 2009), and so the length of a wave does not fully correspond with the length of an oestrus cycle.

The length of a follicular wave varies across individual females (Skidmore et al., 1996a; Manjunatha et al., 2012), but individual animals usually exhibited similar wave patterns and repeatability (Manjunatha et al., 2012). According to the study by Alfuraiji (1999), only 37.5% of females underwent two follicular cycles within 60 days of research, the same proportion of females had one cycle, and no cycle occurred in 25.0% of females.

The signs of oestrus include vocalization, frequent urination, tail raising, staying close to the male and behaving submissively to him, mounting of other females (Aboulela, 1994; Skidmore et al., 1996a; Alfuraiji, 1999; Vyas and Sahani, 2000), but these behavioural signs of oestrus in female camels are weak and have little connection to their ovarian activity, because they are not always associated with the presence of a dominant follicle. Sometime females showed signs of oestrous but were not in oestrous and vice versa. Moreover, males often prefer to mate females which are not exhibiting visible oestrus (Skidmore et al., 1996a; Vyas &

Sahani, 2000). In contrast to humans, camel bulls recognize female in heat well (Vyas and Sahani, 2000), and that is why the camel bull is used for oestrous detection (Zhao et al., 1994).

Unfortunately, other aspects of camels' reproduction are not so easy. Spontaneous ovulation was also observed in non-mated camel females (Nagy et al., 2005; Manjunatha et al., 2012) and so a short luteal phase is sometimes present (Marie and Anouassi, 1987; Skidmore, 2005). The occurrence of spontaneous ovulation was higher in lactating females than in non-lactating (14.3% vs. 1.4% of all follicular waves in the group, respectively) (Nagy et al., 2005). But in contrast to other species with induced ovulation, pseudopregnancy was not detected in camels (Marie and Anouassi, 1987).

2.2.5. Copulation and Ejaculation

Copulation is performing in a sitting (sternal) position (Figure 3) (Merkt et al., 1990; Franklin, 2011) and it is accompanied by rutting displays of the mating bull (Franklin, 2011). The females are usually relaxed and ruminating (Franklin, 2011).

The average duration of copulation is 5 to 6 minutes with a range between 2.5 and 20 minutes (Agarwal and Khanna, 1993; Zhao et al., 1994; Skidmore et al., 1996a; Hemeida et al., 2001; Mosaferi et al., 2005). After ejaculation, the male drops down next to the female (Merkt et al., 1990).

Average ejaculate volume varies from 4.3–8.2 ml with a range between 0.1 and 26.0 ml (Agarwal and Khanna, 1993; Zhao et al., 1994; Skidmore et al., 2000; Hemeida et al., 2001; Deen et al., 2003; Mosaferi et al., 2005; Skidmore and Billah, 2006; Wani et al., 2008). The biggest volume can be reached during the peak of breeding season (Deen et al., 2003). Average semen pH is 7.3 (7.1–7.9) (Agarwal and Khanna, 1993; Zhao et al., 1994; Mosaferi et al., 2005).

Figure 3: Mating.

The usual sperm concentration is from 414 to 566 million per ml (Agarwal and Khanna, 1993; Mosaferi et al., 2005) but the mean concentration was found to be lower in some farms, between 150 and 350 million per ml (Skidmore et al., 2000; Deen et al., 2003; Skidmore and Billah, 2006; Wani et al., 2008). In Bactrian camels, the average concentration was counted to be 559 million per ml (Zhao et al., 1994). The percentage of live spermatozoa is 59.1 ± 0.5

(Agarwal and Khanna, 1993). The number of spermatogonia, spermatocytes, spermatids and spermatozoa differs according to the season; the highest numbers are in the late winter and spring (Abdel-Raouf et al., 1975), i.e. in breeding season.

2.2.6. Pregnancy

Ovulation occurs 72 and 96 hours post-mating (Skidmore et al., 1996a; Vyas and Sahani, 2000). Similarly to cats or rabbits, camels have ovulation induced by mating (McKinnon et al., 1994; Zhao et al., 1994). Levels of luteinising hormone increase after mating (Marie and Anouassi, 1986) and it is followed by ovulation and formation of the corpus luteum (CL) on the ovary (Marie and Anouassi, 1986; Skidmore et al., 1996a). The majority of pregnancies occur in the left horn of the uterus (ElWishy, 1988; Srikandakumar et al., 2001). CL can be located in the right or left ovary, so the migration of an ovum from the right ovary to the opposite horn is common in camels. Multiple CL were also observed in 6.7% of cases, but twins almost never occur thanks to high embryonic mortality (Tinson et al., 2001; Gordon, 2004). The mean size and weight of CL are 22 mm and 4.8 g (Wilson, 1984; ElWishy, 1988).

Evidence on the length of a gestation varies across the studies. The average is between 370 and 385 days with a range from 348 to 403 days. Some studies present a range from 324 to 426 days (Al Mutairi, 2000; Musa et al., 2000; Skidmore et al., 2000; Musa et al., 2006; Al-Sobayil, 2008; Nagy and Juhasz, 2008). Agarwal et al. (1987) found significant differences between the length of gestation of camels carrying male and female foetuses (398 ± 13 vs. 372 ± 11 days, respectively) (Agarwal et al., 1987). Gestation was found to be slightly longer in Bactrian camels, with an average from 402 to 416 days (Zhao et al., 1994; Zhao et al., 1998).

2.2.7. Parturition

Five to ten days before parturition, a slight swelling of the udder and vulva is visible. About three to five hours before calving, the female becomes agitated and avoids other herd members. Interrupting feeding, lying down and standing up is also common (Wilson, 1984; Bhakat, 2006). Increased aggressiveness, looking at the flank, raking the ground with the feet and the presence of a discharge from the vulva was also observed (Bhakat, 2006). Loud vocalisation was also observed by the author of this PhD thesis in some females. The female sometimes walks away from the herd to calve, but parturition in the middle of the herd is also quite common. Calving usually takes place in a sitting position or lying on the side (Wilson, 1984; Bhakat, 2006). Standing during calving is rare, but also reported (Abdussamad et al., 2011). The complete length of parturition with all processes takes about 6 hours (Elias and

Cohen, 1986). Females prefer to give birth during the day (Al-Sobayil, 2008) and according to Nagy and Juhasz (2008), over half of parturitions occur in the afternoon.

The whole process of parturition was well described by Bhakat (2006). The calf is usually delivered in 25 minutes after the appearance of its front legs. The calf is followed by the placenta after an average of 52 minutes (Bhakat, 2006). Maternal behaviour is described in next chapter. The calf usually stands up after multiple attempts within one to one and half hours and tries to suckle milk (Elias and Cohen, 1986; Bhakat, 2006).

Sometimes inexperienced females ignore or reject their calf and keepers usually force them to adopt it (Schwartz et al., 1992; Puschmann et al., 2013), but in the author's experience, is better to separate the mother and calf into smaller stables and monitor their interactions and suckling. When the female exhibits no aggression and the calf is active, it usually reaches the udder within one day and a bond is created between mother and calf.

The involution of the uterus occurs 25–30 days after parturition (Vyas and Sahani, 2000). The first oestrus occurs 14–30 days after calving (Wilson, 1984; Abdel Rahim, 1989), especially when the female is well fed (Yagil and Etzion, 1984), and can be mated again at this time (Degen et al., 1987).

2.2.8. Maternal and Suckling Behaviour

Almost no scientific papers focus on the maternal behaviour of camels. Some descriptive information can be found in older review articles or books, but these sources are again based on other papers and don't deal with empirical data (see for example Wilson (1984) or (Fayed and Matoock, 1996). Some aspects of maternal behaviour were described by Bhakat (2006).

After parturition, the female does not look after her young one intensively. The mother stimulates the calf to stand up by nibbling at the calf's head or by kicking the calf with her front legs (Puschmann et al., 2013). Camels do not lick or clean their young ones (Yagil, 2006; Puschmann et al., 2013) but smell them (Bhakat, 2006) and communicates with calves by specific vocalisation (Koláčková, 2008; Franklin, 2011; Puschmann et al., 2013). Camels do not eat their placenta. The majority of females adopt their calves immediately after parturition, but some of them need a longer time. Once a bond is created, the female protects her calf intensively (Bhakat, 2006).

Calves usually form little groups (Gauthier-Pilters, 1971). New-born calves suckle milk once to three times per hour (Bhakat, 2006). Calves older than five months suckle milk 4-12 times per day. The stage of lactation affects suckling frequency, which decreases with the age of the calf. 80% of suckling bouts are initiated by the calf (Simpkin et al., 1997). According to Packer et al. (1992), allosuckling does not occur in Camelids, but our observations confirmed a quite high incidence of allosuckling (i.e. when a calf suckles a non-maternal female) and intersucking (i.e. when an adult animal suckles milk) in captive Bactrian camels (Haberová and Koláčková, 2010; Brandlova et al., 2013). See detailed study in Chapter 4.3. Zapata et al. (2009a;b) found that allosuckling occurs in other camelids, captive and also wild guanacos (*Lama guanicoe*).

2.2.9. Lactation and Milk Production

The length of lactation varies between 270 to 525 days. The average milk yield during this period in milked animals is from 1,250 to 3,650 litres (about 11 litres per day) (Aujla et al., 1998) but under good management, the average daily yield can reach up to 18 l/day (Khan and Iqbal, 2001). A peak in milk yield is reached by the fourth month of lactation (Gaili et al., 2000). Milk secretion of the camel female decreases with the increasing interval between milk suckling or milking (Alshaikh and Salah, 1994; Simpkin et al., 1997).

Colostrum is produced during the first week after parturition (Konuspayeva et al., 2010b). The composition of camel colostrum and milk varies across the studies. Colostrum of camel is high in protein (about 13.1–14.2%) early after parturition and it decreases over time to an average level of about 2.7–3.6%. Lactose content is relatively similar in colostrum and mature milk (in average about 3.6–5.4%). Fat content is very low in the early colostrum (0.2%) and increases to average levels of about 3.1–7.9% (Zhang et al., 2005; Konuspayeva et al., 2010b). The compositions of the colostrum and mature milk of dromedary and Bactrian camels in different regions are presented in Appendix 2.

The content of vitamin B6 and B16 is similar to that in domestic cattle (Zhang et al., 2005); vitamins A, B1 and B2 are lower but vitamin C is higher in camels (Farah et al., 1992; Zhang et al., 2005). Vitamin E is at lower (Zhang et al., 2005) or similar values to that in domestic cattle (Farah et al., 1992).

No significant differences in mature milk composition during different months or stages of lactation were found (Guliye et al., 2000; Iqbal et al., 2011). Parity also had almost no effect on the main components of camel milk; only the dry matter was significantly lower in females after nine or more parturitions (Guliye et al., 2000). However, milk composition is affected by the season of the year; specifically by the amounts of nutrients and water in diet (Konuspayeva et al., 2010a). The breed of camel also affects the milk yield and composition (Gaili et al., 2000).

2.2.10. Calf Rearing

The birth weight of new-born calves usually ranges from 32 to 42 kg in all camel species (Degen et al., 1987; Agarwal et al., 1992; Sahani et al., 1998; Al Mutairi, 2000; Zhao et al., 2000; Nagy and Juhasz, 2008). Al Mutairi (2000), Zhao et al. (2000) and Nagy and Juhasz (2008) did not find any significant differences between the weights of new-born male and female calves, but in another study males weighed more than females (Sahani et al., 1998). Primiparous females usually have smaller calves then multiparous ones (Sahani et al., 1998; Al Mutairi, 2000). Some calves can weigh less than 25 kg; these calves need more care than calves with a standard weight (Al Mutairi, 2000). The weights of new-born camel calves under various conditions are compared in the study by Kadim et al. (2012).

Male calves have greater gains than female calves (Sahani et al., 1998; Zhao et al., 2000). Camel calves grow more slowly than those of cattle (Kadim et al., 2012). The mean weight of a six-month-old calf is 146.9 ± 0.92 kg and at 36 months 320.9 ± 2.60 kg (Sahani et al., 1998). The average weight gains of camel calves are between 300 and 1,000 g/day (Kadim et al., 2012).

Calf mortality up to one year of age occurred in the range from 3.3 to 31.4% (Abbas et al., 1992; Abbas et al., 2000; Kaufmann, 2000; Kaufmann, 2005; Kalla et al., 2008), depending on many factors. In some years, the annual calf mortality reached 87% (Kaufmann, 2000). The deaths are mostly caused by diseases (mainly diarrhoea), sometimes by drought (up to 22%) or predation (9%) (Kaufmann, 2000). In some areas, death caused by hypothermia is a serious problem in dromedary calves (Al Mutairi, 2000)

The sex ratio of calves (males : females) from mothers under traditional management and sophisticated breeding programs was 1.00 : 1.14 and 1.31–1.36 : 1.00, respectively (Al Mutairi, 2000; Musa et al., 2000; Nagy and Juhasz, 2008).

Calves are weaned at the age of 8–18 months (Aboulela, 1994; Aujla et al., 1998; Farah et al., 2004; Musa et al., 2006). Earlier weaning before seven months of age, i.e. before the next breeding season, is also possible (Aboulela, 1994). Moreover, camel calves can be weaned much earlier (even 30 days after birth) when they are reared with artificial milk (Degen et al., 1987).

Artificial rearing of a calf is time-consuming but feasible. Fostering to a non-maternal camel female in lactation is sometimes possible (Coventry, 2002); some camel females tolerate the suckling of non-filial calves (Brandlova et al., 2013). Colostrum can be obtained from the mother; in untamed camels it is possible to use colostrum from the cow of a domestic cattle or goat. The daily intake of milk should be about 10–15% of the calf's body weight (Puschmann et al., 2013). Mature milk can also be obtained from a camel, cow or goat, or a milk replacer can be fed (Coventry, 2002). While commercial milk replacers for camels are only available in some localities, milk replacers for cattle or a customised replacer can also be used (Haberová and Fedorov, 2012). Detailed methods for artificial calf rearing were described by Coventry (2002) or Haberová and Fedorov (2012) (see Chapter 4.4.).

2.2.11. Monitoring of Reproductive Status in Female Camels

Rectal palpation in a sitting (Banerjee et al., 1981; Deen et al., 2005) or standing position (Tibary et al., 2001) is quite a common method of examining follicles and pregnancy confirmation. For pregnancy diagnosis, rectal palpation is effective 60 days after mating (Vyas and Sahani, 2000). Vaginal examination is also possible (Banerjee et al., 1981).

Transrectal ultrasonography in a standing or sitting position (Nowshari and Ali, 2005; Skidmore and Billah, 2006; Nikjou et al., 2008) or endovaginal ultrasonography (Vyas and Sahani, 2000) is an increasingly common method in recent years. Pregnancy diagnosis by transrectal ultrasonography is usually done at 15 to 20, 60 and 90 days after mating or artificial insemination (Skidmore et al., 2000; Khatir and Anouassi, 2006). The embryonic vesicle, embryo and the heartbeat of the embryo can be imaged 18, 23, and 30 days after mating, respectively (Vyas et al., 2002; Ali et al., 2013). Rectal ultrasonography can be also used for evaluating female ovarian activity (Skidmore et al., 1996a), monitoring foetal development and prenatal sex determination (Ali et al., 2013).

Blood progesterone is another useful tool for early pregnancy diagnosis (Bakheit et al., 2008). When the progesterone level is above 1 ng/ml 15 days after mating, the female can be determined to be pregnant (Zhao et al., 1994). However it is recommended to confirm pregnancy later as well due to the quite high percentage of embryonic deaths in camels (Nagy and Juhasz, 2008). Milk progesterone can also be measured for pregnancy diagnosis, and a bovine milk progesterone kit can be used (Abdel Rahim and Elnazier, 1987).

According to the study by Banerjee et al. (1981), the urine of pregnant camel cows was dark yellow and in non-pregnant was light yellow to yellow. The urine pH was more alkaline (8.3 ±0.25) until day 80 of gestation than in non-pregnant females (7.4 ± 0.37). Its specific gravity is higher in pregnant cows (1.086 ± 0.003) until 60 days of pregnancy than in non-pregnant (1.036 ± 0.01) (Banerjee et al., 1981). The Cuboni reaction of urine seems to be usable in camels (El-Ghannam et al., 1974); see Chapters 2.3.1. and 4.1. for details.

The pregnant female had significantly lower values of blood protein, albumin, iron, and calcium, and significantly higher alkaline phosphatise (Saeed et al., 2009), but these indicators are not used for pregnancy diagnosis.

Females in heat can be detected by camel bull (Zhao et al., 1994). Tail cocking of the female is sometimes used for pregnancy diagnosis (Abdussamad et al., 2011; Muhammad et al., 2011), but the accuracy of this method is debatable (Skidmore, 2000).

2.2.12. Hormonal Changes

The hormonal changes in serum or plasma have already been well documented in camels. While oestradiol and progesterone levels were often used for monitoring the reproductive cycle and pregnancy in dromedary camels (Skidmore et al., 1996a, b), the number of studies focusing on Bactrian camels is considerably lower; but we noticed an increasing trend in recent years (Zhao et al., 1994; Zhao et al., 1998). The hormonal profiles of both species are comparable.

The hormonal levels in camels change not only according to the oestrous cycle, they are also affected by the season and the age of the animal (Yagil and Etzion, 1980; Agarwal and Khanna, 1993; Al-Qarawi et al., 2000; El-Harairy and Attia, 2010). Levels of oestradiol-17ß, progesterone, luteinizing hormone and follicle-stimulating hormone were determined in camels by radioimmunoassay (RIA) (Agarwal et al., 1987; Agarwal et al., 1992; Skidmore et al., 1996a; Alfuraiji, 1999; Al-Eknah et al., 2001; Zhao et al., 2001; Deen et al., 2007; Hussein et al., 2008) or by enzyme-linked immunosorbent assay (ELISA) (Nagy et al., 2005; Nikjou et al., 2008; Ali et al., 2010; El-Harairy and Attia, 2010). The extraction of hormones is quite often required when RIA is used. Progesterone is extracted with petroleum ether and oestradiol-17ß by diethyl ether or ethyl acetate extraction (Yagil and Etzion, 1980; Zhao et al., 1998; Al-Eknah et al., 2001). The usage of fluoroimmunoassay is quite rare, but feasible (Ayoub et al., 2003).

2.2.12.1. Hormonal Changes in Non-pregnant Females

Oestradiol (E2) levels rise with the increasing size of follicles (Skidmore et al., 1996a; Hussein et al., 2008), when the values fluctuates between 10 and 55 pg/ml (Bono et al., 1989; Ayoub et al., 2003; Deen et al., 2007; Hussein et al., 2008). The E2 level reaches its highest values, between 25 and 92 pg/ ml, during oestrous (Skidmore et al., 1996a; Ayoub et al., 2003; Deen et al., 2007; Hussein et al., 2008). In some cases, the peaks in E2 can reach up to 400 pg/ml (Deen et al., 2007).

P4 concentrations remain under a level of 0.5 ng/ml during heat and three to four days after ovulation (if it occurred). When mating and subsequent ovulation took place, the levels increase with the presence of the corpus luteum and achieve a peak with a range between 0.79 and 2.9 ng/ml 8–10 days after heat. The values then drop again quickly below a level of < 0.5 ng/ml (Skidmore et al., 1996a; Ayoub et al., 2003; Deen, 2008).

The concentration of serum luteinizing hormone (LH) fluctuates during the cycle between 0.2 to 1.15 ng/ml, and increase to its highest levels of 1.2 to 2.38 ng/ml on the day of mating or artificial insemination (with the occurrence of mature follicles) (Bono et al., 1989; Hussein et al., 2008). Zhao et al. (2001) reported values at this time of up to 20.3 ± 18.8 ng/ml.

Follicle-stimulating hormone (FSH) levels increase in parallel with the diameter of middle size follicles, then gradually decrease with the presence of larger follicles (Hussein et al., 2008). During breeding season, testosterone level increases in camel females as well (El-Harairy et al., 2010).

However the hormonal level can also be influenced by some reproductive disorders such as the occurrence of cysts or vaginal adhesions (Hussein et al., 2008; Ali et al., 2010).

2.2.12.2. Hormonal Changes in Pregnant Females

The mean E2 levels increase from a basal level of 20 pg/ml to a level of around 40 pg/ml in 50 days of pregnancy and to 90 pg/ml at 90 days of pregnancy. The levels of about 100 pg/ml (with fluctuations between 66.72 ± 36.22 to 184.8 ± 102.48 pg/ml) remain until 300 days of pregnancy. After that, the E2 concentration increases to its maximal values (450–650 pg/ml) in the last month of pregnancy. A big decline to levels of about 200 pg/ml occurs on the day of parturition, and it deceases to 30–45 pg/ml one day later (Agarwal et al., 1987; Skidmore et al., 1996b; Zhao et al., 1998; Ayoub et al., 2003). The highest reported E2 level during camel pregnancy was 1212.97 pg/ml (Zhao et al., 1998).

The corpus luteum is essential for the maintenance of pregnancy. When the P4 concentration falls during pregnancy to under 1 ng/ml, an abortion occurs (Zhao et al., 1998; Deen et al., 2005). The ovaries together with the CL are the main source of progesterone (Al-Eknah et al., 2001). The progesterone level stays under a level of 0.5 ng/ml in the first three days after ovulation. The P4 concentration increases above 2 ng/ml and reaches values of about 3–5 ng/ml 14 days after mating and ovulation. It is possible to confirm pregnancy by this time if the P4 level is above 1 ng/ml. Non-pregnant animals have lower P4 values; usually below 0.5 ng/ml. During the remainder of the pregnancy, the concentration varies between 2 and 6 ng/ml. Sometimes, P4 can reach values of up to 12.5 ng/ml. The concentration starts to decrease two months before parturition and then drops rapidly to a level below 2.5 ng/ml one day before parturition (Zhao et al., 1994; Skidmore et al., 1996b; Zhao et al., 1998; Ayoub et al., 2003; Bakheit et al., 2008). One day after parturition, the P4 concentration fluctuates between 0.5 and 3.2 ng/ml and then declines gradually to an undetectable level over the next 8 days (Agarwal et al., 1992; Zhao and Chen, 1995).

Two peaks of oestrone sulfate were noticed during camel pregnancy. The peaks reach average values of 9.6 ng/ml and 11 ng/ml 40 and 70 days after ovulation, respectively. Oestrone sulfate together with PGFM (prostaglandin F2 alpha metabolite) rise to 46.0 ng/ml and 1,000.0 pg/ml, respectively, before parturition (Skidmore et al., 1996b).

According to Agarwal et al. (1987), camel females bearing a male foetus had overall lower oestradiol (76.5 ± 10.8 pg/ml) and higher progesterone levels (5.13 ± 0.69 ng/ml) than females with a female foetus (112.3 ± 19.6 pg/ml and 3.45 ± 0.28 ng/ml, respectively).

2.2.12.3. Hormonal Changes in Males

The total plasma androgens level varies during the year, and the highest occurs during breeding season (Yagil and Etzion, 1980; Bono et al., 1989). Testosterone also significantly increases in breeding season, when it reaches values of about 1.9 to 2.72 ng/ml, compared to values of about 1.0 ng/ml during the non-breeding season (Zia Ur et al., 2007; El-Harairy and Attia, 2010). Testosterone in adult camels can reach up to 4.8 ng/ml (Al-Qarawi et al., 2000). These androgens are also excreted via the secretion of the poll gland in breeding season. The androgen concentration in this secretion was about 36 ng/ml (Yagil and Etzion, 1980).

Progesterone level increases during breeding season in males as well (203.19 ± 14.87 vs. 150.75 ± 10.12 pg/ml in non-breeding season), but no significant seasonal changes in E2 were found (15.79 ± 1.29 vs. 13.2 ± 1.58) (Zia Ur et al., 2007). A new ovulation hormone was discovered in 2001 in the seminal plasma of males. It was called “ovulation-inducing factor” (OIF) (Pan et al., 2001).

2.2.13. Reproductive Performance

The reproductive performance of camels under traditional management is often very poor (Tibary et al., 2005). This is usually due to late puberty, a long calving interval, delayed post-partum mating, poor nutrition and a high incidence of calf death (Yagil and Etzion, 1984; Abbas et al., 1992).

The first oestrous in females occurs at an average age of 39.2 months (Zhao et al., 1994; Musa et al., 2006). The age at first calving depends on the breeder’s experience, region and the camel breed. According to available studies, the first calving occurred at an average

age of 48 months in China (Zhao et al., 1994), 52 months in Sudan or Saudi Arabia (Abbas et al., 2000; Musa et al., 2006), 55.3 months in UAE (Aboulela, 1994), 58.4 to 68.4 months in Kenya, depending on the camel breed (Kaufmann, 2005), and 60 to 72 months in Pakistan (Aujla et al., 1998). In some traditional breeding systems, camel females give birth even later, e.g. the mean age of the female at the first calving was over six years in Nigeria or Sudan (Abbas et al., 1992; Kalla et al., 2008).

The average calving (parturition) interval in traditional systems is 21–29 months (Kohler-Rollefson et al., 1991; Abbas et al., 1992; Aboulela, 1994; Zhao et al., 1994; Aujla et al., 1998; Kaufmann, 2005; Musa et al., 2006; Bakheit et al., 2008; Kalla et al., 2008) and in some cases longer (35 months) (Musa et al., 2000). The calving interval can be shortened to 17–20 months in semi-intensive breeding systems (Abbas et al., 2000; Bakheit et al., 2008). It is evident that enhanced feeding and care can improved reproductive performance (Bakheit et al., 2008). Some females under professional management can have a calf almost every year (Kohler-Rollefson et al., 1991).

Conception rate from natural mating in animals under traditional management is usually between 40% and 45% (Vyas and Sahani, 2000; Bakheit et al., 2008; Kalla et al., 2008), but the conception rate was reported to be higher in Chinese Bactrian camels; up to 60% (Zhao et al., 1994). Camel females in semi-intensive breeding systems had an annual conception rate of about 68%, or 88% in the 18-month evaluation (Abbas et al., 2000; Bakheit et al., 2008). The conception rate is lower (about 40%) in females younger than 5 years or in females older than 15 years (Abbas et al., 2000). Camels in a professional breeding program can reach an annual conception rate of about 86.3% (Nagy and Juhasz, 2008). The per-cycle conception rate can be influenced by the month of mating. The highest rate, over 50%, can be reached during the peak of breeding season (Nagy and Juhasz, 2008). Repeated mating is often needed in camels (Aboulela, 1994).

The incidence of early embryonic death can reach 10.2% and usually occurs between 33 and 97 days after conception. But more than half of the females can become pregnant again in the same breeding season (Nagy and Juhasz, 2008). Abortion rates are usually between 6.3% and 12.1% (Zeleke and Bekele, 2000; Kaufmann, 2005; Nagy and Juhasz, 2008) but it can be much more higher, over 25%, when pregnant females are allowed to eat poisonous plants or were infected with brucellosis (Kalla et al., 2008). In contrast, some studs had an abortion rate of only 3.8% (Abbas et al., 2000).

Twins in camels are very rare. Although the presence of more dominant follicles and multiple ovulations are quite common in camels (ElWishy, 1988; Manjunatha et al., 2012), only sporadic reports about live-born camel's twins can be found because the majority of these pregnancies ended with embryonic death or abortion (Tinson et al., 2001; Gordon, 2004).

Females can be reproductively active up to the age of 22 years (Kohler-Rollefson et al., 1991). Camel females usually have 6 calves by the age of 15 years, only some females have 8–10 calves (Kohler-Rollefson et al., 1991; Abbas et al., 1992). But camel females older than 20 years can still reproduce as well (Mukasa-Mugerwa, 1981; Merkt et al., 1990).

Reproductive efficiency is affected by biological and pathological factors, but the influence of human factors and breeding management also play an important role in the successful breeding of camels (Tibary et al., 2005). Uterine infections and fibrosis, ovarian hydrobursitis (accumulation of fluid in ovarian bursa), and persistent luteal structures are among the most common causes of camel female infertility (Tibary et al., 2001; Ali et al., 2011). "Hand mating" is a common practise in camel breeders who detect a female in heat by the presence of oestrous behaviour (Abdel Rahim and Elnazier, 1987; Tibary et al., 2001; Abdussamad et al., 2011) but as reported earlier, the behavioural signs of oestrus are not often connected with the presence of a mature follicle (Skidmore et al., 1996a; Vyas & Sahani, 2000), so this could also be a common cause of reproductive failure. Moreover, Marie and Anouassi (1986) suggested that repeated mating could be essential for the maintenance of high LH concentrations and successful ovulation. Tibary et al. (2005) summarised the factors which affect reproductive efficiency.

2.2.14. Interspecific Hybrids and Assisted Reproductive Technologies

All Camelids have 74 chromosomes, so it is possible to interbreed them (Skidmore et al., 1999; Hoffman and Baum, 2006). Crossbreeds between dromedary and Bactrian camels are probably the most common hybrids. Experiments with the hybridisation of camels were done in the former USSR (todays Russia and the surrounding countries) to obtain hardy animals with high milk and meat yields (Baimukanov, 1989) but some recent studies also exist (Nurseitova et al., 2014). Hybrids between Dromedary and Bactrian camels are strong animals, with one long hump. Crossbreeds are also used in Turkey for camel wrestling (Yilmaz et al., 2011). On the other hand, hybridisation with domestic Bactrian camels presents a severe threat for the Wild Bactrian camels (Tulgat and Schaller, 1992).

Modern reproductive technologies in camel breeding are being used more and more, but the development of techniques which are commonly used in other husbandry animals is more complicated in camels, and progress has been very slow (Gordon, 2004). Semen collection and preservation, artificial insemination and hormonal induction or synchronisation of ovulation are quite often practiced; see for example study of Agarwal et al. (1997); Deen et al. (2003); Deen et al. (2005); Al-Sobayil (2008), But freezing of embryos and embryo transfer have also been successfully performed with camels (Skidmore et al., 2000; Skidmore and Billah, 2011). Besides this, interspecies embryo transfers were also successful, and the first calf of a Bactrian camel was delivered by a dromedary female (Niasari-Naslaji et al., 2009). In the last twenty years, several experiments to hybridize camels with llamas (*Lama* spp. and *Vicugna* spp.) have been conducted. The first interspecific live-born hybrid, called cama, resulted from the artificial insemination of a guanaco female with the sperm of a dromedary camel (Skidmore et al., 1999). Research into interspecific hybridisation and embryo transfer in camelids is still ongoing (Skidmore et al., 2001; Niasari-Naslaji et al., 2014). Moreover, the first cloned dromedary camel was born in 2009 (Wani et al., 2010).

2.3. Non-invasive Diagnostic Tools and their Application is Camels

The management of reproduction and pregnancy diagnosis are very important and decisive for the successful breeding of every species in captivity (Ramsay et al., 1994; Kleiman, 2010). In zoological gardens, camels and other ungulates are rarely trained and every handling activity and medical examination, including blood sampling or other invasive procedures needs physical or chemical restraint, which is dangerous and stressful (Christman, 2010).

Non-invasive diagnosis and the monitoring of hormones are increasingly popular, and these methods enable samples to be collected very often without causing pain and stress to the animal. Moreover, hormonal levels in urine and faeces seem to be more resistant to temporary disturbances (Monfort, 2003).

2.3.1. Diagnosis from Urine

Monitoring hormonal levels from urine is usually a quick and inexpensive method (Monfort, 2003). Urine testing can be useful not only for pregnancy diagnosis, but also for monitoring the basic state of health of the animals (Czekala et al., 1990; Ganswindt et al., 2002; Vaden et al., 2011). Urinary pH can be used for monitoring nutritional disorders (Roby et al., 1987; Nappert and Naylor, 2001) or post parturient diseases (Markusfeld, 1987).

The urinary pH of a healthy camel is alkaline and ranges between 7.4 and 10.0 (Banerjee et al., 1981; Khogali, 2005; Al-Bashan, 2011), which is similar to the values for other ruminants (Allen and Borkowski, 1999; Kaneko, 2014). Its specific gravity ranges from 1.022 to

1.086 (Read, 1925; Banerjee et al., 1981; Amer and Alhendi, 1996), however Al-Bashan (2011) reported a specific gravity of only 1.005 to 1.015.

While urinary steroid hormones monitoring is often unfeasible for routine application among breeders, the Cuboni reaction is quite a commonly offered test with mares. The test is based on the fluorescent reaction of urinary estrogens with benzene, hydrochloric acid and sulfuric acid, and it is a useful qualitative method for pregnancy diagnosis in mares (*Equus caballus*) during the second half of gestation (Bates and Cohen, 1950; Cox and Galina, 1970; Wolfsdorf, 2009). According to the preliminary study by El-Ghannam et al. (1974), who tested the Cuboni reaction in slaughtered camel females, it is possible to get a positive Cuboni reaction from females bearing a 26 cm long foetus, which corresponds to 4.5 months of pregnancy (Musa, 1977). Very good results can be obtained from the 7th month of pregnancy (El-Ghannam et al., 1974; Musa, 1977). No further study has been published.

The barium chloride test was formerly used in the diagnosis of pregnancy in the cows of domestic cattle (Rao and Veena, 2009) and sows (Ndu et al., 2000; Lalrintluanga and Dutta, 2009). The addition of a few drops of 1% barium chloride solution into the urine caused a white precipitate in non-pregnant animals. In pregnant animals, the precipitate is not formed (Ndu et al., 2000). According to reviews by Khanvilkar et al. (2009b) and Purohit (2010), the BaCl test is applicable in camels, but no scientific articles describing this method and its results in camels were found.

2.3.2. Diagnosis from Saliva

Despite the fact that diagnosis from saliva is to some degree controversial and for some authors unreliable, some studies consider this method suitable (Gröschl, 2008). The number of studies using salivary assays is showing an increasing trend (Chiappin et al., 2007). The collection of saliva is easy, especially in domestic and tamed animals, but it is also applicable in half-tamed zoo animals which are trained for hand feeding (Chiappin et al., 2007; Gröschl, 2008).

Camels are able to produce a large amount of saliva (Wemmer and Murtaugh, 1980; von Engelhardt et al., 2006; Haberová et al., 2012) and they are usually accustomed to feeding by hand in zoological gardens, so these facts demonstrate the great potential of saliva usage as a diagnostic specimen in camels (see Figure 4). But to the author’s knowledge, there have been no studies focusing on hormonal changes in camels kept in Europe, nor in the saliva of camels anywhere.

Monitoring hormonal changes through saliva has already been confirmed e.g in buffaloes (*Bubalus bubalis*) (Qureshi et al., 1999), black rhinoceros (*Diceros bicornis*) (Czekala and Callison, 1996) or Indian rhinoceros (*Rhinoceros unicornis*) (Gomez et al., 2004). Common commercial kits for hormonal assays from the saliva of humans are also suitable for ungulates. For example, these hormonal analyses were used in ponies and domestic cattle in the laboratories of Salimetrics Europe Ltd. Even when some samples were quite contaminated with food, the tests showed good results (Sparrow, 2010).

Besides salivary hormonal assays, saliva crystallization is also used for monitoring the different reproductive stages, although this phenomenon was not often studied in animals. The crystallization is also connected with oestrogen levels. When the level of oestrogens is high (in the second half of pregnancy or during oestrus) the fluid crystallizes into a fern pattern, when the level is intermediate into an arboriform (club-moss) pattern, and when the level is low into dotted or no formations (Rob and Stehlík, 1983). Oestrogens increase and progesterone reduces the occurrence of crystallization (Tsiligianni et al., 2001). Pardo-Carmona et al. (2010) verified saliva crystallization in bitches. We also confirmed saliva crystallization in Bactrian camels and domestic cattle (Haberová, 2010; Skalova et al., 2013).

Figure 4: Sampling of camel saliva.

3. AIMS OF THE THESIS

This thesis aimed to investigate the reproduction, maternal behaviour and rearing of Bactrian camels (*Camelus bactrianus*) kept in zoological gardens of Central Europe.

Four studies related to the aim of the thesis were published:

1) **Application of Noninvasive Pregnancy Diagnosis in Bactrian Camels (*Camelus bactrianus*) Using Cuboni Reaction and Barium Chloride Test**

The aim of the study was to examine alternative, cheap and non-invasive methods of pregnancy diagnosis from urine in camels which could be easily obtainable for camel keepers, usable in practise and applicable in half-tamed camels bred in zoological gardens. We tested whether the barium chloride test and the Cuboni reaction are applicable in camels with at least a high accuracy during the last third of pregnancy.

2) **Salivary Sex Steroid Hormones in Female Bactrian Camels (*Camelus bactrianus*) during Different Reproductive Stages**

The aim of this study was to verify the potential of a steroid hormones assay from saliva as a new non-invasive method which had never been used in camels, and to determine the differences in salivary oestradiol and progesterone levels in pregnant and non-pregnant camel females. A further aim was to evaluate the seasonal changes in non-pregnant female camels kept in European zoological gardens.

3) **Camel Calves as Opportunistic Milk Thefts? The First Description of Allosuckling in Domestic Bactrian Camel (*Camelus bactrianus*)**

This study aimed to analyse maternal and suckling behaviour in captive camels. Since the occurrence of allosuckling in captive camels was observed in our research, the milk theft hypothesis was tested with several predictions: 1) a calf would suckle from the non-maternal dam standing in an other than antiparallel suckling position; 2) the calf would preferably join the filial calf when suckling the non-maternal dam; 3) termination of a non-filial suckling bout by the dam will be more frequent for an antiparallel position than for a lateral position; 4) the incidence of allosuckling will increase with age of the allosuckling calf; 5) the duration of a suckling bout will be shorter with non-filial than filial calves.

4) Umělý odchov velblouda dvouhrbého (*Camelus bactrianus*) – Hand-rearing of Bactrian camel (*Camelus bactrianus*)

Since the PhD thesis author had the opportunity to assist and actively manage two cases of artificial calf rearing in the Prague zoological garden, the aim of this paper was to describe her experiences with the artificial rearing of camel calves and summarise camel rearing in this zoological garden.

4. MATERIAL AND METHODS

The theses was following recommendation of the Methodical Manual for the MSc Theses Writing of the Faculty of Tropical AgriSciences (FTA), Czech University of Life Sciences (CULS) Prague (FTA, 2014a); no exact structure rules or manual for PhD thesis writing exists at FTA CULS Prague. References were cited according to the Citation Rules of the FTA, CULS Prague (FTA, 2014b).

The results are based on four published scientific articles. Detailed description of animals and methods used can be found in the articles included in Chapter 5.

5. RESULTS

5.1. Application of Noninvasive Pregnancy Diagnosis in Bactrian Camels (*Camelus bactrianus*) Using Cuboni Reaction and Barium Chloride Test

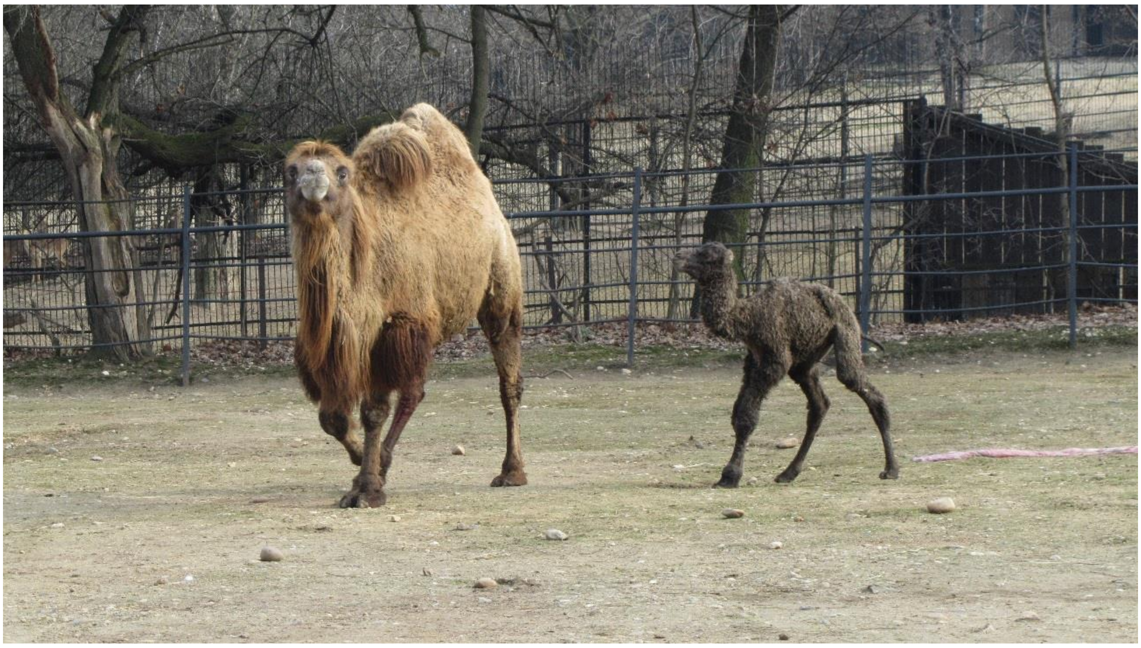

Figure 5: Camel female with her new-born calf.

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APPLICATION OF NONINVASIVE PREGNANCY DIAGNOSIS IN BACTRIAN CAMELS (*CAMELUS BACTRIANUS*) USING CUBONI REACTION AND BARIUM CHLORIDE TEST

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Abstract: Pregnancy diagnoses in half-tamed animals are often very complicated. This study aimed to examine the alternative noninvasive and cheap methods of pregnancy diagnosis from urine in domestic Bactrian camels (*Camelus bactrianus*). Urine from 14 female camels kept in four European zoologic gardens was collected and tested by two chemical tests—Cuboni reaction and barium chloride test. The Cuboni reaction was significantly ($P < 0.01$) affected by the pregnancy status of female camels. The total accuracy of the Cuboni reaction was 70.5% but it increased significantly ($P < 0.05$) in the time leading up to parturition. The accuracy was 100% in the 3rd third of pregnancy. Urine of nonpregnant females did not react with a solution of barium chloride while, contrary to other studies, white precipitates formed mostly (80 to 100%) in urine of pregnant females. This study concluded that the Cuboni reaction is applicable for pregnancy diagnosis in camels.
Key words: BaCl test, camel, gestation, urine, zoo.

BRIEF COMMUNICATION

The domestic dromedary (*Camelus dromedarius*, Linnaeus, 1758) and Bactrian camel (*Camelus bactrianus*, Linnaeus, 1758) are frequently bred in zoos,[8] from where specific needs for noninvasive pregnancy diagnosis emerged. Camels are seasonal breeders and their ovulation is induced by mating.[14,15] The length of estrus cycle in female camels (*Camelus* spp.) varies from 18 to 32 days[14,15] and the gestation period in Bactrian camels is 402.22 ± 11.5 days.[15] The estrus behavior is not always associated with the presence of a dominant follicle,[13] and common methods of pregnancy confirmation in tamed camels, such as rectal palpation, are not usually applicable to half-tamed camels kept in European zoos.

The Cuboni reaction, based on a fluorescent reaction of urine estrogens with benzene, hydrochloric acid, and sulfuric acid, is a useful qualitative method for pregnancy diagnosis in horse mares (*Equus caballus*) during the second half of gestation.[3] A preliminary study on the Cuboni reaction applied to the urine of slaughtered female camels was tested by El-Ghannam et al.,[7] and the reaction seemed applicable from the seventh month of gestation.

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The barium chloride (BaCl) test was formerly used in pregnancy diagnosis for cows (*Bos taurus*)[10] and sows (*Sus* sp.).[9] The addition of a few drops of 1% BaCl solution into the urine causes a white precipitate in nonpregnant animals. In pregnant animals the precipitate is not formed.[10]

The aim of this research was to examine the alternative and cheap noninvasive methods of pregnancy diagnosis using the urine of camels kept in the zoos. A total of 14 adult female Bactrian camels housed in Central European zoos were included in the research. Two females stopped their reproduction after second parturition for unknown reasons and have not been pregnant in recent years; see Table 1 for details.

The urine of female camels was collected in a disposable, 0.5-L plastic PET cup mounted by wire to a broom handle between September 2010 and November 2011. At least two samples from each female were collected during this period. The interval between sampling for one animal was at least 1 mo.

Urine was stored in the refrigerator at a temperature of 6–8°C and tested 1–5 days after sampling. The urine was left to reach room temperature before testing. The Cuboni reactions were carried out in the State Veterinary Institute Prague, Czech Republic, by the standardized method that is commonly used for horse mares. A urine sample with a minimum volume of 20 ml was required for this test. The results were classified as positive (animal is pregnant), negative (animal is not pregnant), and dubious (result is not clear). Thirteen samples were tested twice with the Cuboni reaction, and both results from

Table 1. Camel females included in the study.

Female ID	Facility[a]	Date of birth	No. of samples from pregnant stage	No. of samples from nonpregnant stage	Sampling period (days before parturition)	Notes
1	PRG	29 Jan 1993	7	8	370–117	
2	UNL	9 Aug 1993	3	0	281–141	Twins, abortion
3	PLZ	24 Mar 1994	4	0	111–63	
4	PRG	26 Jun 1987	9	4	215–6	
5	PRG	9 Mar 1997	0	3		Stopped reproduction
6	BRA	8 Mar 2000	2	1	396	
7	UNL	2003	3	0	273–133	
8	UNL	2003	2	0	262–171	
9	PRG	2 June 1998	10	0	380–42	During two pregnancies
10	UNL	20 Mar 1989	0	1		Stopped reproduction
11	BRA	1981	0	2		
12	PRG	15 Mar 1995	7	7	396–143	Abortion
13	UNL	2001	0	2		
14	BRA	22 Jun 1995	3	0	203–119	
Summary			50	28	396–6	

[a] BRA, Bratislava; PLZ, Plzeň; PRG, Prague; UNL, Ústí nad Labem.

these samples were included in the statistical evaluation.

The BaCl test was conducted in the laboratory of the university. Drops of 1% solution of BaCl were added to a 5-ml sample of urine, and the presence of white precipitate was evaluated after addition of 1, 5, 10, and 15 drops of BaCl solution. Presence of white precipitates was evaluated as negative (animal was evaluated as nonpregnant), and the absence of white precipitates in the end of experiment was evaluated as positive (animal was evaluated as pregnant).[9,10]

Final evaluations of all measurements and tests were compared with real pregnancy status of camel females at the end of the calving period, and the accuracy of both tests was counted retrospectively from the term of parturition. Two females ended the pregnancy by abortion. In one of these females, the time of possible parturition was estimated according to crown vertebral rump length (CVRL) of the aborted calf.[2] The second case of abortion was caused by bearing of twins. Because twins are smaller than calves of single births,[6] it was not effective to measure CVRL. The length of gestation was estimated by the zoologist according to his experiences and animal management knowledge.

The thirds of camel pregnancy were set similar to Bello et al.:[2] 130 days or less to parturition were classified as 3rd third, 131–260 days as 2nd third, and 261 days or more as 1st third. Periods of the year were set according to meteorologic reckoning: spring (March–May), summer (June–August), autumn (September–November), and winter (December–February).[1]

Data distribution was tested by the Shapiro–Wilk test. Data lacked normal distribution and the nonparametric statistics (Wilcoxon signed-rank test, Kruskal–Wallis test, Spearman's rank correlation, median test, and Pearson chi-square [χ^2] test) were used. The significance level was accepted at $P < 0.05$.

The total accuracy of the Cuboni reaction was 70.5% ($n = 78$). The percentage of false-positive results and false-negative results from all Cuboni reactions was 16.7 and 9.0%, respectively. The results of Cuboni reactions were significantly affected by pregnancy status of females (Pearson χ^2: 18.9325, df = 6, $P = 0.0043$). The accuracy of the Cuboni reaction was 100% in females in the 3rd third of pregnancy. A detailed overview of the results of the Cuboni reaction is provided in Table 2. The general accuracy (true or false result of the test) increased in the time leading up to parturition (Spearman's rank correlation: $r = -0.2817$; $P < 0.05$).

The Cuboni reactions in two females who had ended their pregnancies by abortion were significantly more-often a false negative than in other pregnant females (Pearson χ^2: 13.6324, df = 2, $P = 0.0011$). The accuracy was significantly affected by the period of the year (Pearson χ^2: 17.3537, df = 9, $P = 0.043$). Dubious reactions occurred only during autumn while false negative results occurred only in spring and autumn. The differences between observed and expected frequencies showed that more true results were reached

Table 2. The results of the Cuboni reactions.

Result of Cuboni reaction	Nonpregnant	1st third of pregnancy	2nd third of pregnancy	3rd third of pregnancy
Negative	50.00%[a]	23.53%	17.65%	0.00%
Positive	46.43%	76.47%[a]	70.59%[a]	100.00%[a]
Dubious	3.57%	0.00%	11.76%	0.00%
No. of samples	28	17	17	16

[a] The true results of the test.

during summer while more false-positive results were reached during winter.

A trend in relationship between results of the BaCl test (n = 25) and real pregnancy status of animals was found, but the differences in results were not significant (Pearson χ^2: 7.13245, df = 3, P = 0.068). The results of the test (Table 3) showed reverse results in comparison with other studies. The overall percentage of false-positive results and false-negative results from all reactions was 36.0 and 40.0%, respectively.

The accuracy of pregnancy tests based on chemical reactions of estrogens in urine is limited; nevertheless, they can be useful for pregnancy confirmation in wild and untamed ungulates.[12] The 100% accuracy of the Cuboni reaction was possible to get after 230 days of pregnancy in camels.[7] The accuracy in earlier phases and in nonpregnant camels was lower than in horse mares.[11] False-negative results were significantly connected with abortions, but the majority (57.1%) of false-negative results occurred during the 1st third of pregnancy when false negative results are expected.[11] The last false-negative result was reached 160 days before parturition in a female that delivered a healthy calf.

False-positive results of the Cuboni reaction obtained from nonpregnant females suggested that the urine of females in estrus can react positively. Blood estradiol fluctuates during the estrous cycle in camels between 10–55 pg/ml[4] and reaches the highest values between 25–92 pg/ml during estrus.[4,13] However, in some cases peaks of estradiol in camels can achieve values up to 400 pg/ml,[4] and some female camels in heat could reach the total estrogen levels similar to pregnant horse mares between the 90th and 150th days of pregnancy.[5] Estrus occurs in camels mainly during winter and spring months,[1] so it seems that the best time for the Cuboni reaction is in summer when females do not go into a heat.[1] Summer is also usually a late-enough season to get a positive reaction from pregnant females. In combination with repeated sampling during autumn, the breeder would be able to get more-accurate results.

Opposite results in the BaCl test were obtained in comparison to other studies.[9,10] In this study, urine of nonpregnant females did not react with the solution of BaCl very often, while white precipitates formed in 80–100% of cases in the urine of pregnant females.

The results confirmed that the Cuboni reaction is applicable for pregnancy diagnosis in female camels. The best times for sampling in the northern hemisphere are summer and autumn, the seasons which ensure a higher accuracy of the Cuboni reaction. The barium chloride test needs further inspection to be applicable for pregnancy diagnosis in camels.

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Table 3. The results of the barium chloride test.

Result of BaCl test	Nonpregnant	1st third of pregnancy	2nd third of pregnancy	3rd third of pregnancy
Negative	30.77%[a]	100.00%	83.33%	80.00%
Positive	69.23%	0.00%[a]	16.67%[a]	20.00%[a]
No. of samples	13	1	6	5

[a] The true results of the test.

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5.2. Salivary Sex Steroid Hormones in Female Bactrian Camels (*Camelus bactrianus*) during Different Reproductive Stages

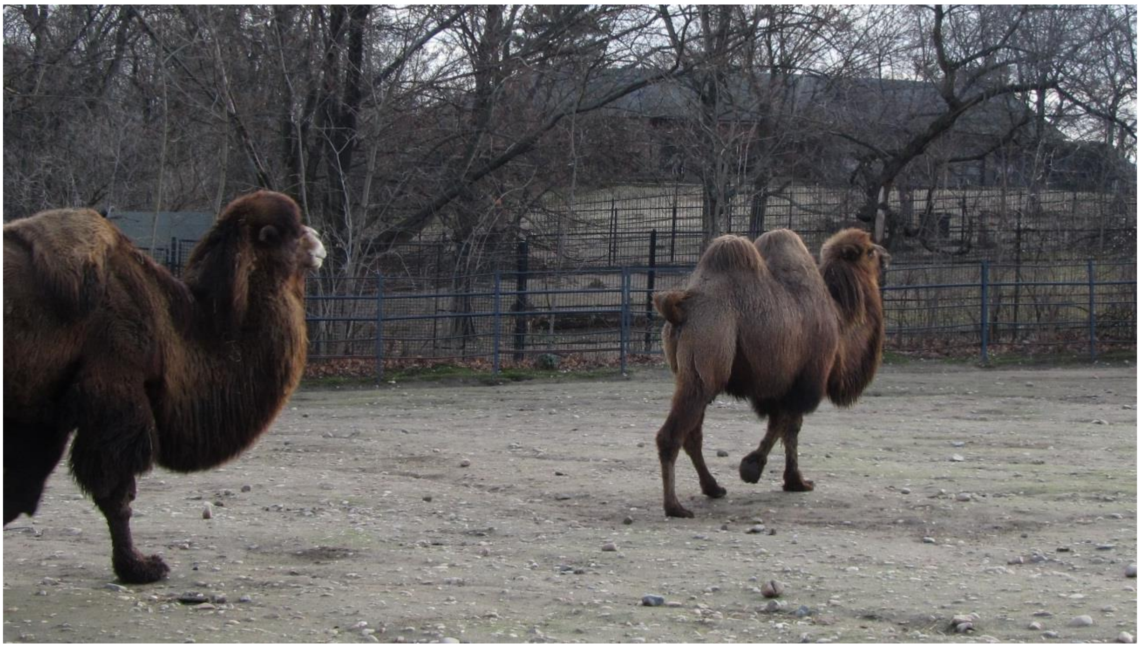

Figure 6: Tail curling is not very precise method for pregnancy diagnosis; this female is not pregnant.

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SALIVARY SEX STEROID HORMONES IN FEMALE BACTRIAN CAMELS (*Camelus bactrianus*) DURING DIFFERENT REPRODUCTIVE STAGES

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ABSTRACT

The study aimed to verify the usage of salivary sex steroid hormones monitored in captive bactrian camels (*Camelus bactrianus*) as a new non-invasive method in this species. Saliva of 5 adult female camels housed in the Prague zoological garden were sampled for more than 1 year in maximum interval of 10 days and concentrations of progesterone (P_4) and oestradiol (E_2) were measured. The concentrations of P_4 (n = 312) and E_2 (n = 310) were both significantly ($p < 0.0001$) affected by pregnancy status of animals. Mean (±SE) P_4 concentrations in non-pregnant stages were 2.234 (±0.220) nmol/l, while during the 2[nd] third of pregnancy it was 5.105 (±0.858) nmol/l. E_2 concentrations differed significantly between non-pregnant stages with mean value 0.037 (±0.005) nmol/l and during the 3[rd] third of pregnancy when reached 0.098 (±0.012) nmol/l. The seasonal differences in non-pregnant female camels were also evaluated. While no significant seasonal deviations were found in E_2 concentrations, P_4 values were significantly higher in summer than in spring. The study concluded that salivary P_4 and E_2 measurements are suitable for monitoring different reproductive stages in half-tamed female camels. The autumn and winter seasons seemed to be the best for pregnancy diagnosis in camels bred in Europe.

Key words: Camelid, oestradiol, pregnancy, progesterone, saliva

All studies focused on hormonal changes in camels were carried out so far only from blood, e.g. study of Elias *et al* (1984), Zhao *et al* (1994), Skidmore *et al* (1996a, 1996b), or from milk (Abdel Rahim and Elnazier, 1987; Abdel Rahim, 1989). The hormonal changes in serum or plasma have been already well documented in female camels. Oestradiol, also reported as oestradiol-17β, (E_2) and progesterone (P_4) concentrations were often used for monitoring the reproductive cycle and pregnancy in dromedary camels (*Camelus dromedarius*) (Skidmore *et al,* 1996a, 1996b; Ayoub *et al,* 2003; Muhammad *et al,* 2011) but also in bactrian camels (*Camelus bactrianus*) (Zhao *et al,* 1994; Zhao *et al,* 1998) with similar results. Nevertheless, camels are seasonal breeders (Al-Hazmi, 2000, Nowshari and Ali, 2005; Ali *et al,* 2008; El-Harairy and Attia, 2010; El-Harairy *et al,* 2010) and hormonal changes in non-pregnant animals can also be influenced by a season of the year (Agarwal and Khanna, 1993; Al-Qarawi *et al,* 2000; El-Harairy and Attia, 2010). The breeding season depends on area of distribution, climate, temperature, humidity, day light length and rainfalls (Bono *et al,* 1989; El-Harairy and Attia, 2010). The average gestation period is between 370 and 385 days in dromedary camels (Skidmore *et al,* 2000; Musa *et al,* 2006; Al-Sobayil, 2008) and 402.22 ± 11.5 days in bactrian camels (Zhao *et al,* 1994).

Camels are able to produce large amount of saliva (Haberová *et al,* 2012; Wemmer and Murtaugh, 1980; von Engelhardt *et al,* 2006) and monitoring of reproduction through saliva has been already confirmed in other ungulate species, e.g. in buffaloes (*Bubalus bubalis*) (Qureshi *et al,* 1999), black rhinoceros (*Diceros bicornis*) (Czekala and Callison, 1996) or Indian rhinoceros (*Rhinoceros unicornis*) (Gomez *et al,* 2004).

The aim of the study was to verify the potential of steroid hormones monitoring in camels from saliva as a new non-invasive method in bactrian camels and evaluate the seasonal changes in non-pregnant female camels kept in European zoological garden.

Materials and Methods

Animals and husbandry

Five adult female bactrian camels (*Camelus bactrianus*) housed in the Prague zoological garden, Czech Republic, aged between 14-24 years were included in the research. Females were kept together

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with male for the whole time and these were able to mate freely, so the time of conception was not known. Each third of pregnancy was defined retrospectively from parturition and it is represented according to respective days based on overall gestation length. The term of parturition was used for data analysis. At least ⅓ out of every detected pregnancy (n = 5) was monitored during the sampling period. Non-pregnant stages were noticed in 4 females.

Animals were fed by meadow hay, fresh vegetable, pellets for herbivores, and oats for the majority of year. Fresh fodder was provided during some parts of spring. Animals were trained for human contact, enabling touching and feeding from hand but another type of manipulation was not possible.

Sample collection and hormonal assay

Samples were collected from 3rd February 2011 to 27th February 2012. The interval between samplings was 2 or 3 days during the period between December and March, 7 days during April, May and November, and 10 days between June and October.

The whole herd was lured close to the fence by showing a bucket with dried bread. Usually, whole herd ran up to the sampling place immediately and animals were showing their willingness to cooperate. The sampling was carried out through the bar fence. In selected animal, the production of saliva was stimulated by feeding a piece of dried wheat or wheat-rye bread without any pieces of salt or cereals. When the camel was trying to get to the bread, saliva was wiped off from the tongue by the handle of a disposable plastic spoon. Saliva was collected in the crease of the spoon handle and was removed to the polypropylene centrifuge tubes of volume 15 ml. The process was repeated several times until the amount of foamy saliva reached 5 ml. The animal was rewarded by piece of bread.

The test tubes with saliva were stored in temperature between 4 – 8°C and transported to a freezer (-20°C) within 12 hours after the sampling. The concentrations of P_4 and E_2 were measured in the Institute of Endocrinology (Prague, Czech Republic). The total unconjugated 17β-oestradiol was measured by the RIA Spectria Estradiol kit® (Orion Diagnostica Oy, Finland) and P_4 by the Progesterone RIA kit (Immunotech, A Beckman Coulter Company, France). The salivary samples were processed according to the guidelines of the kits' producers and by the same methods as samples of serum.

All animals included in the research cooperated of their own free will and they were not forced to sampling. The research was approved by the management of the Prague zoological garden and also by the veterinarian of this institution.

Data analysis

Stages of pregnancy were counted from the term of parturition. One pregnancy ended by abortion and the term of possible parturition was counted from Crown Vertebral Rump Length (CVRL) of the aborted calf (Elwishy *et al*, 1981; Bello *et al*, 2012; Hena and Sonfada, 2012). The thirds of camel pregnancy were based on the study of Bello *et al* (2012): 130 days or less to parturition were classified as 3rd ⅓, 131 to 260 days to parturition as 2nd ⅓ and 261 days or more as 1st ⅓.

Periods of the year were classified according to meteorological reckoning: spring (March, April, May), summer (June, July, August), autumn (September, October, November), and winter (December, January, February) (Barnett and Dobson, 2010).

The statistics were performed using software Statistica CZ 12 (Stat Soft, Inc.). The data distribution was not normal ($p < 0.01$; Lilliefors test and Shapiro–Wilk test) and the non-parametric statistics (Kruskal-Wallis test, Multiple comparison of p-values, Mann–Whitney U test) were used to analyse the results. The significance level was accepted at $p < 0.05$.

Low, undetectable concentrations of P_4 (n=16) were obtained mainly (75.0%) in non-pregnant animals. E_2 concentration under detectable level was noticed only in one sample. These samples were analysed as zero concentrations. Two samples overreached a maximum measurable E_2 concentration and these samples were excluded from the analyses.

Results

312 samples of camel saliva were collected and successfully analysed. The concentrations of P_4 (n = 312) and E_2 (n = 310) were both significantly ($p < 0.0001$) affected by pregnancy status of animals (Kruskal-Wallis test: H (4, N = 313) = 27.1809 and H (4, N = 311) = 39.3600, respectively). Mean P_4 and E_2 levels during different stages of reproduction are provided in Table 1.

Hormonal changes during pregnancy

Mean P_4 concentrations are shown in Fig 1. P_4 concentrations fluctuated during the pregnancy and the peak, averaging 6.06±2.90 nmol/l, was reached between the 150th and 126th day before parturition (BP). The mean P_4 concentrations fell to 3.61±0.75 nmol/l by the last 50 days of pregnancy.

As shown in Fig 2, the E_2 concentrations increased rapidly 175 days BP and reached the 1st peak, averaging 0.115±0.060 nmol/l, between the 150th and 126th day BP. The E_2 increased significantly (Mann-Whitney U Test, $U = 447$, $p < 0.01$) again 50 days BP and reached the values of 0.120±0.022 nmol/l in the last 25 days of pregnancy.

Hormonal changes in non-pregnant animals during the year

In non-pregnant animals, the P_4 values were significantly affected by the season (Kruskal-Wallis test: $H (3, N = 114) = 10.2520$, $p = 0.0165$). P_4 concentrations were significantly higher in summer than in spring (Multiple comparison of p-values, $p = 0.0345$) and as shown in Fig 3, similar trend visible for the winter season (Multiple comparison of p-values, $p = 0.0522$). The concentrations during autumn and winter stayed always under 6.5 nmol/l.

P_4 concentrations differed significantly also between months (Kruskal-Wallis test: $H (11, N= 114) = 27.0522$, $p = 0.0045$) and the highest mean concentrations were reached in July and August (4.248 and 5.055 nmol/l). The lowest mean P_4 concentrations were noticed during February (1.159 nmol/l).

However, no significant seasonal or monthly differences ($p > 0.05$) were found in E_2 concentrations.

Discussion

The study is the first report of salivary sex steroid hormone monitoring in camels and also the first study focused on hormone monitoring in camels bred in Europe. Despite the fact that the number of studies focused on hormonal analysis from saliva in animals is limited and concentrations of hormones in saliva are fluctuating (Hofman, 2001; Kobelt *et al,* 2003), the presented results showed that salivary P_4 and E_2 are suitable for monitoring of different reproductive stages in half-tamed female camels similar as in other ungulate species, e.g. study of Czekala and Callison (1996), Moriyoshi *et al* (1996), Qureshi *et al* (1999) and Gomez *et al* (2004).

Mean P_4 and E_2 concentration changes during the pregnancies in presented study had similar pattern as in study of Skidmore *et al* (1996a) or Zhao *et al* (1998) dealing with blood samples of camels. A positive correlation between salivary and serum

Table 1. Salivary progesterone and oestradiol concentrations in female camels during different phases of reproduction.

	Progesterone (nmol/l)		Oestradiol (nmol/l)	
	Mean ± SE	Range	Mean ± SE	Range
Non-pregnant (n = 114)	2.2339±0.2197[a]	0.0000 - 14.4100	0.0374±0.0049[a]	0.0010 - 0.3620
Post-partum period (n = 14)	1.5193±0.3057[a,b]	0.0000 - 3.3600	0.0500±0.0099[a,b]	0.0120 - 0.1630
1st third of pregnancy (n = 66)	2.5527±0.3346[a,b]	0.0000 - 11.6400	0.0564±0.0086[b]	0.0000 - 0.3680
2nd third of pregnancy (n = 29/28)	5.1045±0.8584[c]	0.3600 - 23.3100	0.0693±0.0181[a,b]	0.0060 - 1.6500
3rd third of pregnancy (n = 90/89)	4.3357±0.5348[b,c]	0.0000 - 26.2700	0.0978±0.0118[b]	0.0078 - 0.6149

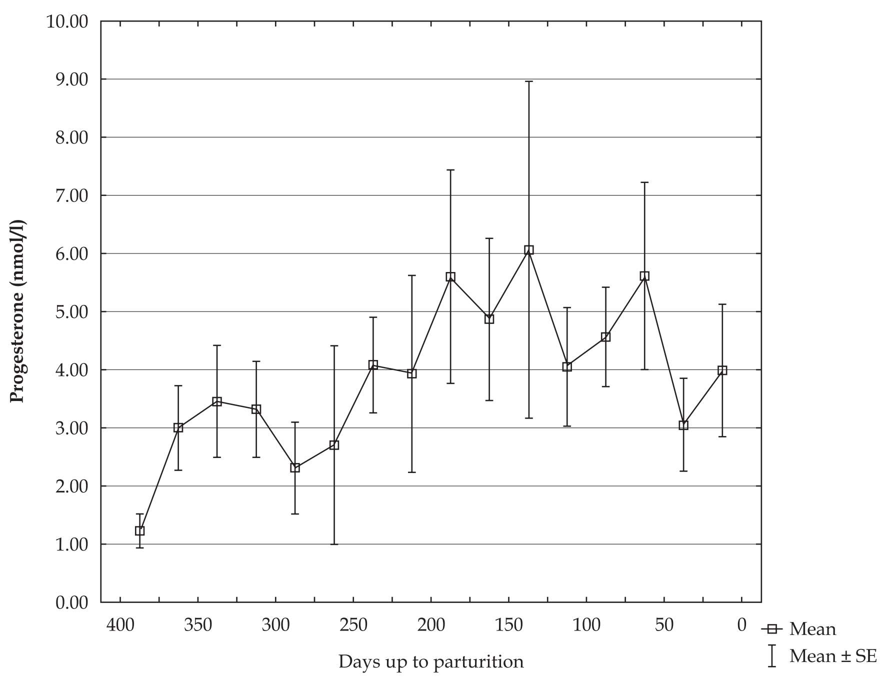

Fig 1. Mean ± SE progesterone concentrations in camel saliva during pregnancy.

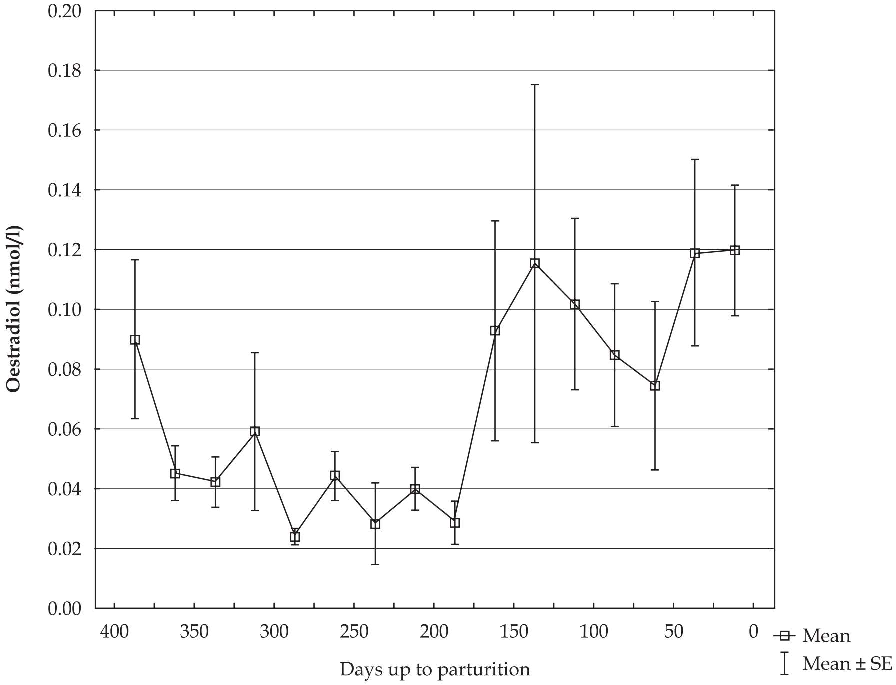

Fig 2. Mean ± SE oestradiol concentrations in camel saliva during pregnancy.

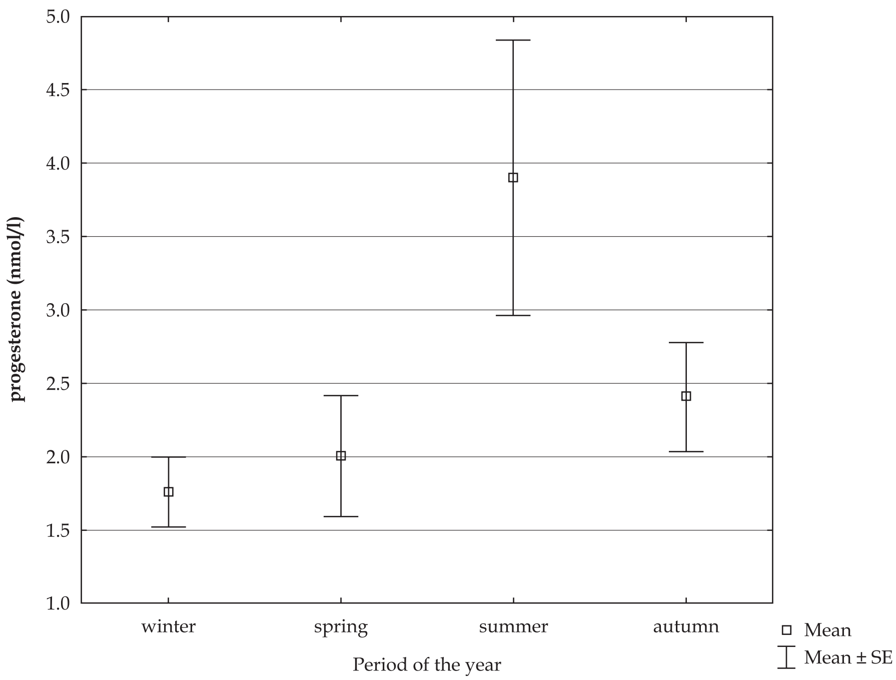

Fig 3. Mean ± SE progesterone concentrations in saliva of non-pregnant female camels during different periods of the year.

oestradiol was already reported by Qureshi *et al* (1999) in buffaloes and the fact that serum hormonal concentrations correlated with concentrations in saliva was well proved in human (Hofman, 2001).

Salivary sex steroid hormones concentrations seemed not to be suitable for early pregnancy diagnosis in camels because no significant differences between pregnant and non-pregnant animals were found. However, with repeated sampling, it could be possible to determine pregnancy during the 2nd or 3rd third of pregnancy. Contrary to research of Volkery *et al* (2012) carried out on alpacas, this study confirmed the significant differences between P_4 concentrations at these stages of pregnancy and non-pregnant animals. Some results evaluated in the first 2 months of pregnancy could also be misrepresented because it was not possible to determine exact time of conception. Higher E_2 concentrations noticed in the beginning of pregnancy (Fig 2), between 375^{th} and 400^{th} day before parturition, were also probably caused by the impossibility to know the exact day of conception and some animals could be in oestrus in that time.

Contrary to Hussein *et al* (2008), seasonal difference in P_4 concentrations were proved non-pregnant animals. Higher P_4 concentrations during the spring and summer could be caused by unrecognised early embryonic losses that are common in camels (Nagy and Juhasz, 2008); some animals could conceived during spring but they did not give a birth due to embryonic loss and they could be incorrectly assessed as non-pregnant.

The majority (89.4%) of parturitions of camels kept in the Prague zoological garden occurred between February and May (Haberová and Fedorov, 2012), so autumn or winter periods belongs usually to 2^{nd} or 3^{rd} third of pregnancy in camels. This fact, together with the finding that P_4 stayed at lower concentrations during autumn and winter in non-pregnant animals instigate to the conclusion that these 2 periods of the year seem to be the best for pregnancy diagnosis in camels in Europe or temperate regions of northern Hemisphere.

Present study did not prove any significant variation in mean values of E_2 in non-pregnant animals between different months or seasons similar like Hussein *et al* (2008) but the maximum fluctuations were reached during February, March, June and December. This feature could be caused to the presence of heat in camel females (Skidmore *et al*, 1996a) which was not possible to detect in presented study and selected breeding facility.

In conclusion, (1) hormonal analyses of saliva are suitable for reproductive status monitoring in female camels and more detailed research at this topic can be recommended. (2) Both salivary steroid hormones, oestradiol-17β and progesterone, can be recommended for pregnancy diagnosis during the 2^{nd} and 3^{rd} third of camel pregnancy. (3) Autumn or winter seems to be the best periods for pregnancy diagnosis in camels in Europe.

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5.3. Camel Calves as Opportunistic Milk Thefts? The First Description of Allosuckling in Domestic Bactrian Camel (*Camelus bactrianus*)

Figure 7: Allosucking in Bactrian camels.

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Camel Calves as Opportunistic Milk Thefts? The First Description of Allosuckling in Domestic Bactrian Camel (*Camelus bactrianus*)

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Abstract

Allosuckling is a situation when a female nurses a non-filial offspring. It was described in various ungulate species; however for camels this is the first description of this behaviour. The aim of the study was to assess the occurrence of allosuckling in captive camels (*Camelus bactrianus*) and to test whether it can be explained as a 'milk-theft' (opportunistic behaviour of calves) or alternatively as an altruistic behaviour of females. During 2005 and 2007, nine camel females and ten calves in four zoological gardens in the Czech Republic were observed. In total, 373 sucking bouts were recorded, from which 32 were non-filial (the calf sucked from the non-maternal female). Allosuckling regularly appeared in captive camel herds. As predicted for the milk-theft explanation, the non-filial calves sucked more often in the lateral position and even did not suck in the antiparallel position at all. The non-filial calves preferably joined the filial calf when sucking but in five cases (15.6% of non-filial sucking bouts) the calves sucked from non-maternal dam without the presence of filial calf. We then expected the differences in terminations of sucking bouts by females but did not find any difference in sucking terminations for filial and non-filial calves. As the calves were getting older, the incidence of allosucking increased. This was probably because skills of the calf to outwit the non-maternal dam increased and/or the older calves might be more motivated for allosucking due to the weaning process. Finally, duration of a sucking bout was shorter with non-filial than filial calves. The results of the study support the hypothesis of 'milk theft', being mostly performed by calves behaving as opportunistic parasites, but we cannot reject certain level of altruism from the allonursing females or their increased degree of tolerance to non-filial calves.

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Introduction

Allonursing or communal nursing, communal suckling, non-offspring nursing in mammals refers to the situation when a lactating female nurses a young which is not her own [1,2]. When an offspring sucks milk from female which is not its mother, we call this allosucking or communal sucking [3,4]. We use the term allosuckling for both behaviours together. This phenomenon can be explained as an extreme form of communal maternal care [5,6], known in various mammalian orders [1]. Nevertheless, the explanations of allosuckling occurrence are diverse across the species and situations and functions of allonursing are not well understood. Allosuckling involves tolerance by nursing females, ranging from kin directed discrimination or social affiliation of females [2,7,8], to a parasitic behaviour of young in which they steal milk without the female's acceptance [1,2]. Motivation of calves for allosuckling is often explained as a compensation of nutritional requirements of the young [9,10], using either the tolerance of females or a milk-theft strategy [9–13]. The explanation of allosuckling as an adaptive behaviour of females involves mostly the kin selection hypothesis [1,2,4,10] in which females nurse preferably the offspring of related females, or the reciprocity hypothesis, when females nurse the offspring of another group member reciprocally [14,15]. The reciprocity hypothesis in general is expected to apply in stable groups of social animals and is therefore connected with social affiliation [8]. In accordance with the compensation theory, females in better body condition may be more tolerant to non-filial calves. In some extreme cases a female actively nurses a non-filial offspring because she does not recognize that the offspring is not her own [16]. Roulin [2] calls this behaviour the misdirected parental care, connecting it with milk-theft. The milk-theft hypothesis [1] predicts that the calf tries to 'steal' the milk from a non-maternal female, but when the female recognizes the calf is not her own, she would refuse to nurse it as observed e.g. in various seal species [17]. This behaviour is found more in overcrowded conditions and is more frequent in captive populations [1,2,18].

The dromedary camel (*Camelus dromedarius*) is mentioned in the list of mammals published by Packer *et al.* [1] as the species with no allosuckling occurrence. However, allosuckling has been described in other camelids. Zapata *et al.* [19] reported incidental allosuckling occurrence in wild guanacos (*Lama guanicoe*) and regular occurrence of allosuckling in captive farmed guanacos as behaviours that were consistent with the milk-theft hypothesis and a compensation theory [20,21].

The wild Bactrian camel (*Camelus ferus*) is now considered like a separate species [22] and is found exclusively in China and Mongolia [23]. No information about the suckling behaviour of wild camels has been published. The Bactrian camels kept in European breeding facilities belong to the domestic form (*Camelus bactrianus*) [24]. Camels are monotocous ungulate species, having only one offspring per litter [25,26]. A female in feral or extensively bred camels usually leaves the herd for parturition, while in captivity is often separated from the herd by keepers. The calf follows the mother for several hours after birth. Within a week the mother and the calf rejoin the herd [27,28]. According to our observations in zoo camels, females which are not separated often give birth surrounded by the other herd members. Camels are seasonal breeders and the calves are born during spring months [25]. In European breeding facilities the breeding season is prolonged and calves are born all over the year with a peak in spring months [24]. Camel calves are nursed up to two years and the female can have a calf every two years [28]. Some of the females may give birth every year [29]. According to the study of Sambraus [30], camel calves sucked 8 times in 24 h period, slighter more during the daylight. Nursing dams did not limit sucking of calves up to 3 months of age, while they frequently terminated sucking bouts of older calves [30].

The aim of this study was to provide the first description of allosuckling occurrence in camels and to test possible hypotheses explaining this behaviour. The kin selection hypothesis did not seem to be a major factor in this study, as the females were not related to each another. Based on the findings of Zapata *et al.* [19,20] on another camelid, the guanaco, we predicted the milk-theft hypothesis be the main cause why the camel calves sucked from non-maternal females. If this was valid we predicted that (i) a calf would suck from the non-maternal dam standing in other than antiparallel sucking position so that it was more difficult for the dam to distinguish the calf's identity or to threat the non-filial calf. (ii) A calf should preferably join the filial calf during sucking non-maternal dam. (iii) If the position served as a tactic not to be recognized or threatened, one would expect termination of a sucking bout involving a non-filial calf in an antiparallel position (if any) by the dam be more frequent than if the non-filial calf was sucking in a lateral position. We also predicted that (iv) the incidence of allosucking will increase with age of the allosucking calf as skills of the calf to outwit the non-maternal dam would increase or the calf will be more motivated for allosuckling. Finally, we predicted (v) duration of a sucking bout will take shorter time with non-filial than filial calves. Alternatively, if the result will not correspond with the milk-theft hypothesis, an altruistic behaviour of females should be taken in account, either in the form of reciprocal help or compensation.

Materials and Methods

Ethic statement

Observations of camels were carried out in zoos mostly from the visitors' area or from the background yards when needed. The observer did not enter animal enclosure and did not affect the behaviour, husbandry, and management of studied animals. The zoo managers were informed and agreed with the research activities.

Animals and husbandry

From 2005 to 2007, we have studied maternal behaviour of Bactrian camels kept in four zoological gardens in the Czech Republic (Praha, Brno, Ostrava, Zlín–Lešná). Nine females (one of them reproduced two times within the observation period) and ten calves (4 males, 6 females), were included in the study. The size of herds ranged between 5 and 11 individuals; including 2 to 3 calves (Table 1). All calves in each herd were sired by the same bull, making them half-siblings to one another. Females were not related to one another, but have lived together most of their lives. All except one female were multiparous. Additional data on calves are presented in Table 2. Each animal was identified individually, according to the shape of humps, hair and facial traits. Age, origin, kinship, and other attributes of females were available according to Animal Record Keeping System (ARKS) records of every zoo (see Table 3 for details).

Camels in all facilities were fed once or twice a day by hay and grasses *ad libitum*, supplemented with grains and vegetables, and *ad libitum* water supply. The animals were kept outdoors, mostly with the access to unheated stables or shelters. The outdoor enclosures of camels in the zoos had mostly grass or sandy surface with a similar space allowance in all cases. Even in larger enclosures camels spent most of the time close to each other and were not dispersed. The daily maintenance of herd was done by the keepers either entering the herd directly or moving animals from the stable to enclosure and back to clean all the space without the direct contact with animals. Females were separated before parturition and joined the rest of the herd after two to 30 days of the calves' life.

Recorded variables

We recorded all occurrences of suckling by *ad libitum* sampling method [31]. Selected activities were directly observed by one observer (Karolína Brandlová). The observations were performed monthly in all studied calves during 7–10 hours a day (0800–1800, 0800–1700, 0900–1800, 0800–1600), depending on locality and season, starting as soon as possible after birth of the second calf in the respective herd and continuing at least 3 months.

For each sucking bout we recorded the identity of the animals, duration of sucking bout, position of sucking calf, which animal terminated the sucking bout (mother, calf, or other). The position of the sucking calf was classified into two classes - antiparallel, when the hind part of the calf was directed toward a cow's head, and lateral, when the calf stands at least in the right angle to the cow's body axis. As the gap between the start of sucking and milk let-down is not documented in camels, we consider all bouts longer than 5 seconds as successful as in other studied species e.g. [4,32,33]. Sucking bout was considered to terminate when it was interrupted for at least 10 seconds.

Assessment and statistics

The data were analysed using Statistical Analysis Systems (SAS) version 9.2. Frequency counts for prediction (i) were analysed by computing chi-square test (PROC FREQ). The output contained cell or cells counts less than 5, hence Pearson exact chi-square was used. For other data we used Generalised Linear Mixed Model (GLMM) for analysing numeric variables (PROC MIXED) or categorical variables (PROC GLIMMIX for binary distribution). To account for repeated measures, all mixed model analyses but one were performed using individual camel 'calf' nested within the 'herd' as a random effect. In unbalanced designs with more than one effect, the arithmetic mean for a group may not accurately reflect response for that group, because it does not take other effects into account. Therefore, we used least-squares-means (LSMEANs) instead. LSMEANs are, in effect, within-group means appropriately adjusted for other effects in the model. LSMEANs were computed for each class and differences between classes were

Table 1. Zoological gardens included in the study with the numbers of camels kept and the number of filial and non-filial sucking bouts in herds.

Zoo	Year	Adults (M, F)	Nursing F	Calves (M, F)	Total SB	Non-filial SB	Non-filial SB (%)
Brno	**2006**	**1,4**	**2**	**0,2**	**81**	**0**	**0,00**
Brno	**2007**	**1,3**	**1**	**0,2***	**26**	**3**	**11,54**
Zlín-Lešná	**2005**	**1,2**	**2**	**2,0**	**58**	**2**	**3,45**
Ostrava	**2006**	**1,7**	**2**	**1,1**	**85**	**16**	**18,82**
Ostrava	**2007**	**1,7**	**3**	**1,2**	**36**	**9**	**25,00**
Praha	**2006**	**1,5**	**2**	**1,1**	**87**	**2**	**2,29**
Total					373	32	**8,58**

(M - males; F- females; SB – sucking bout).
*One of the calves was already weaned by its mother but occasionally sucked from a non-maternal dam.
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tested by t-test. For multiple comparisons we used the post hoc Tukey-Kramer adjustment.

We combined predictions (ii) and (iv) into one GLMM for binary distribution modelling the probability for a calf to suck from non-maternal dam. Fixed effects were 'age of the calf' (a continuous predictor that ranged from 1 to 17 months), 'number of calves' taking part in the sucking bout (a categorical factor with levels 1 to 3), 'nursing females' (a categorical factor with levels 2 and 3 females per herd),'sex of the calf' (a categorical factor with male and female levels), and 'sucking order' (a categorical factor with levels the 1st, the 2nd, and the 3rd calf coming to suck). None of the non-filial calves sucked in antiparallel position, therefore the effect 'Position' (antiparallel or lateral) could not be applied. For prediction (iii), we applied a GLMM for binary distribution modelling the probability for a dam to terminate the sucking bout. Fixed effects were 'relatedness' (filial sucking and non-filial sucking), 'position', 'age of the calf', 'sex of the calf', 'nursing females', and 'birth order' (the birth order of the calf within the season and herd). Primarily we were interested in testing the effect of the 'relatedness' alone and/or in an interaction with 'position'. Given that none of the non-filial calves sucked in the antiparallel position, the effect of 'position' had to be omitted. We also examined various combinations of the other fixed effects (i.e., 'relation', 'age of the calf', 'sex of the calf', 'nursing females', and 'birth order') on the termination of sucking by the dam. For prediction (v) we applied GLMM with duration of the sucking bout as a dependent variable. Fixed effects were 'age of the calf', 'age of the dam' (4 to 17 years), 'number of calves', 'relatedness' in interaction with 'position' and in interaction with 'sex of the calf'.

Results

Over the three years of study (2005–2007; 164 hours within 26 days of observation in total) we have recorded 373 sucking bouts (Table 1). The non-filial sucking represented 8.58% (32 out of 373) of all sucking bouts. In all herds, 50% of calves (5) sucked exclusively from their own mothers, and 50% sucked from both own mother and non-filial cows. Six out of nine (66.67%) cows nursed both filial and non-filial calves. Three cows nursed the filial calf exclusively (Table 3). In individual calves, allosucking ranged from 0 to 100% of all sucking bouts. For individual females, allonursing ranged from 0 to 35% of all nursing bouts. Calves allosucked from the females which had younger calves than the allosucking one. The youngest calf in the herd had never allosucked (Table 2).

(i) Sucking position

Filial calves sucked from their mothers mostly standing in the antiparallel position (62.17% of cases), while non-filial calves suckled exclusively in the lateral position (n = 32, difference Pearson exact chi-square test $p = 3.04 * 10^{-13}$, Figure 1).

(ii) Number of sucking calves

Four non-filial calves were involved in a sucking bout without the presence of filial calves five times (15.6% of cases), standing in a lateral position (Figure 2). In all 27 cases when non-filial calves were allosucking with other calf or calves present, they invariably joined already sucking filial calf.

(iii) Termination of sucking by the dam

Termination of sucking by the dam was not affected by any of the tested factors either when they entered the model alone or in any combination with other factors. Non-filial calves never sucked in anti-parallel position, so we could not test the effect of position to termination. Of the non-filial calves which sucked without a presence of filial calf, sucking was terminated by the calf three times, once by the dam and in one case we did not see who terminated the bout.

(iv) Sucking probability

The GLMM model revealed that the probability for a calf to suck from non-maternal dam was affected by 'age' of the calf ($F(1,358) = 3.96$, $p = 0.047$, Figure 3), and 'number of calves' taking part in the sucking bout ($F(1,358) = 27.50$, $p < 0.0001$). In particular, allosucking was more likely in older calves and with increasing number of sucking calves. 'Nursing females' and 'sex of the calf' were not significant predictors and were dropped from the model.

(v) Sucking duration

The mean ($\pm$ SE) sucking duration was 42.93 ± 2.22 s (range 5–270), the mean duration of filial sucking bout 43.50 ± 2.37 s (range 5–270) and the non-filial sucking bout 36.78 ± 5.47 s (range 5–121).

The GLMM model showed that duration of sucking bouts was dependent on the 'number of calves' taking part in the sucking bout ($F(1,191) = 17.19$, $p < 0.0001$), so the sucking bouts involving more than one calf were longer than those involving just one calf, either filial or non-filial (Figure 4). Duration of sucking bouts was also dependent on a relatedness by position interaction ($F(1,367) = 11.05$, $p = 0.001$), meaning that calves in the antipar-

Table 2. Camel calves included in the study with the number of observed hours.

Zoo	Year	Name	Date of birth	Sex	Mother	Age of calf (months)	Observed hours	No. of sucking bouts	Allo-sucking extent (%)	Allo-sucker	Mean duration ± SE of filial sucking (sec.)	Mean duration ± SE of allosucking (sec.)
Brno	2006	April	2.4.2006	F	Isis	2–5	32	75	0,00	NO	22.48±2.38	
Brno	2006	Gaja	18.4.2005	F	Sulika	13–16	32	6	0,00	NO	50.50±22.41	
Brno	2007	April	2.4.2006	F	Isis	14–17	24	3	100,00	YES		37.33±16.83
Brno	2007	Polednice	10.3.2007	F	Sulika	2–5	24	23	0,00	NO	33.87±6.58	
Zlín-Lešná	2005	Marek	17.5.2005	M	Jade	0–3	22	34	0,00	NO	55.15±10.41	
Zlín-Lešná	2005	Aštar	14.3.2005	M	Klaudie	3–6	22	24	8,33	YES	75.91±15.66	10.00±0.00
Ostrava	2006	2sameček	31.3.2006	M	Vendula	2–6	42,5	30	3,33	YES	62.90±9.03	5.00±0.00
Ostrava	2006	1samička	23.3.2006	F	Čora	2–6	42,5	55	27,27	YES	41.23±5.36	36.80±7.39
Ostrava	2007	2sameček	31.3.2006	M	Vendula	14–16	16	8	50,0	YES	41.25±15.20	58.50±24.15
Ostrava	2007	1samička	23.3.2006	F	Čora	14–16	16	11	45,45	YES	32.50±7.06	43.80±14.45
Ostrava	2007	Kobi	2.3.2007	F	Fatima	2–4	16	17	0,00	NO	46.06±10.05	
Praha	2006	Vanda	18.1.2006	F	Lee	6–8	29	43	4,65	YES	37.15±4.96	17.50±5.50
Praha	2006	Víťa	21.7.2006	M	Rona	0–3	29	44	0,00	NO	54.16±6.63	

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Table 3. Camel females included in the study.

Zoo	Female name	Birthdate	Arrival to present zoo	Parity till 2005	Number of calves till 2005	Parity till 2006	Number of calves till 2006	Parity till 2007	Number of calves till 2007	Allonursing extent (%)	Allonurser
Brno	Isis	2.6.1998	24.6.1999	2	1	3	2	3	2	0.00	NO
Brno	Sulika	6.3.1992	6.3.1992	7	5	7	5	8	6	9.38	YES
Zlín-Lešná	Jade	24.2.1997	3.9.1998	4	4	4	4	5	4	5.56	YES
Zlín-Lešná	Klaudie	3.5.1999	3.5.1999	2	1	2	1	3	2	0.00	NO
Ostrava	Vendula	03.04.2000	30.5.2001	1	0	2	1	2	1	35.29	YES
Ostrava	Čora	02.03.2002	30.5.2003	0	0	1	1	1	1	2.13	YES
Ostrava	Fatima	17.05.1990	27.6.2003	2	0	2	0	3	1	26.09	YES
Praha	Lee	02.06.1998	24.6.1999	2	1	3	2	4	3	0.00	NO
Praha	Rona	15.03.1995	26.4.1996	4	1	5	2	5	2	4.35	YES

doi:10.1371/journal.pone.0053052.t003

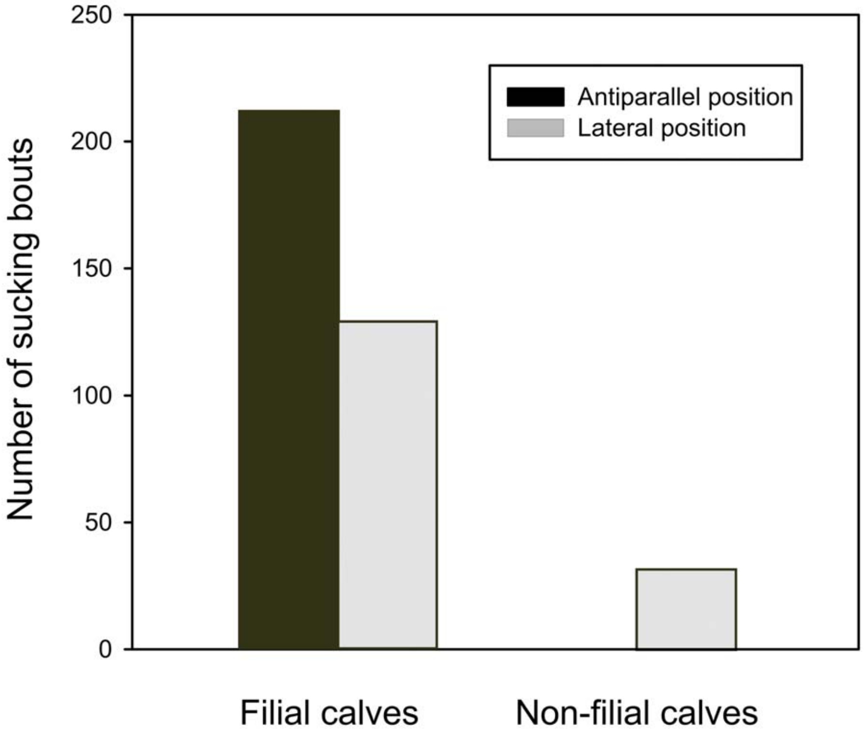

Figure 1. Sucking positions chosen by filial and non-filial camel calves.
doi:10.1371/journal.pone.0053052.g001

allel position (only filial ones) sucked longer than those in the lateral position (either filial or non-filial) (Figure 5 left). Sucking duration depended also on a relatedness by sex interaction ($F(2,20.7) = 3.49$, $p = 0.049$), showing that the sucking bouts of filial males were longer than those of females, both filial and non-filial. Non-filial males did not differ from non-filial females (Figure 5 right). 'Age of the calf' and 'age of the dam' were not significant predictors and were removed from the final model. For non-filial calves only, duration of allosucking was much shorter when the non-filial calves were sucking alone (9±12.54 seconds)

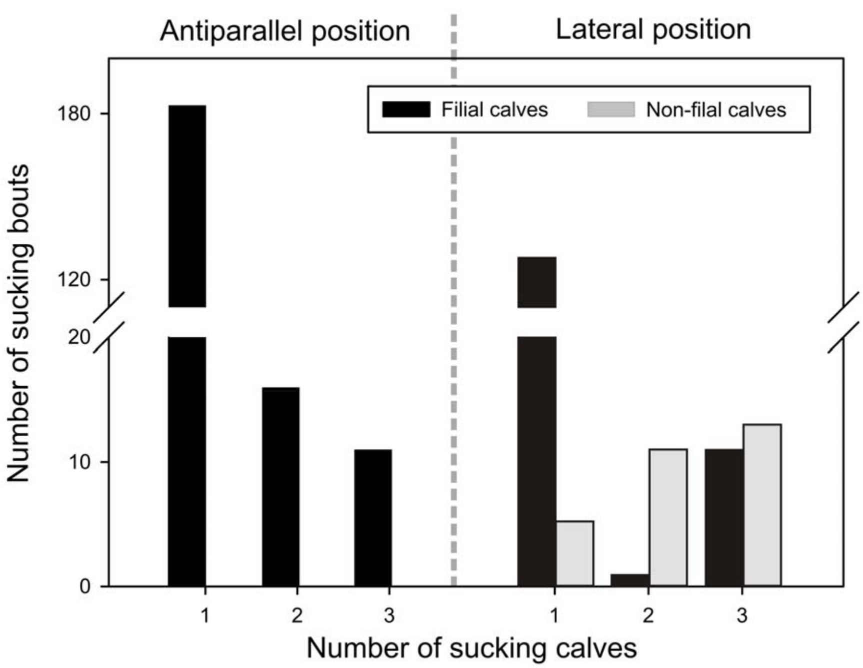

Figure 2. Suckling bout occurrence for filial and non-filial calves according to the number of sucking calves and position during sucking.
doi:10.1371/journal.pone.0053052.g002

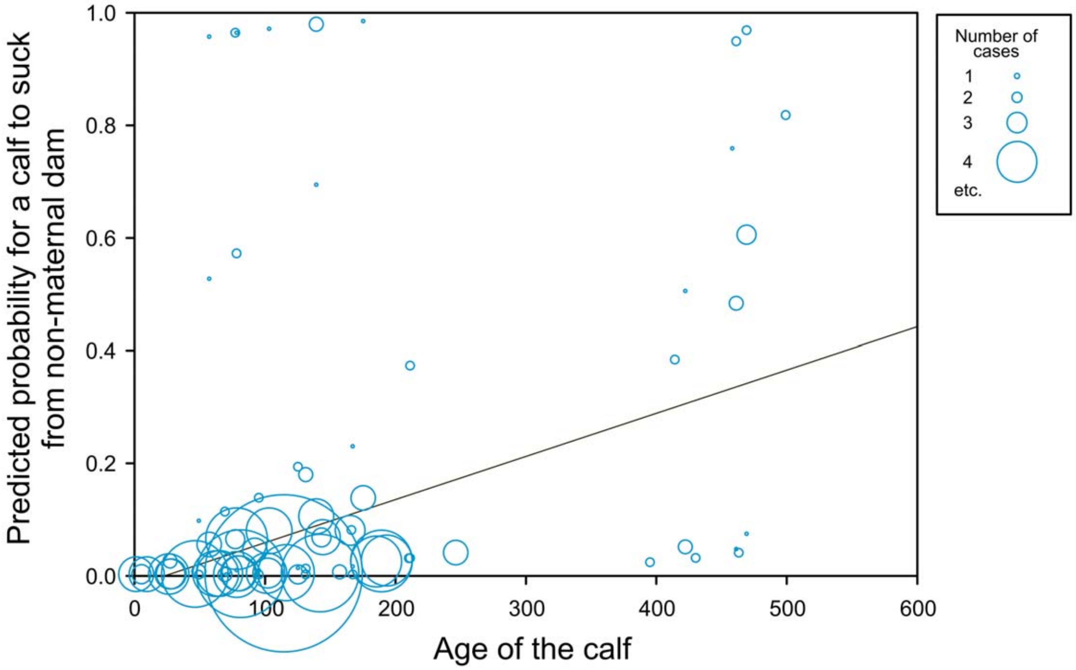

Figure 3. Probability of non-filial suckling bout occurrence according to calf age.
doi:10.1371/journal.pone.0053052.g003

compared when there were one or two other calves (41.92±5.40 seconds, $F(1,32) = 5.82$, $p = 0.02$).

Comparison between allonursing and non-allonursing dams

Comparison between allonursing and non-allonursing dams is shown in Table 3. The numbers of the animals are low for statistical comparison. Nevertheless, none of the characteristics available (age, parity, number of calves reared) seems to play any significant role in whether or not the dam allows non-filial calf to suck.

Discussion

Allosuckling in camels

In this study we brought the first description of allosuckling occurrence in camels. The results have shown that allosuckling occurred in 5 out of 10 calves from 4 camel herds containing more

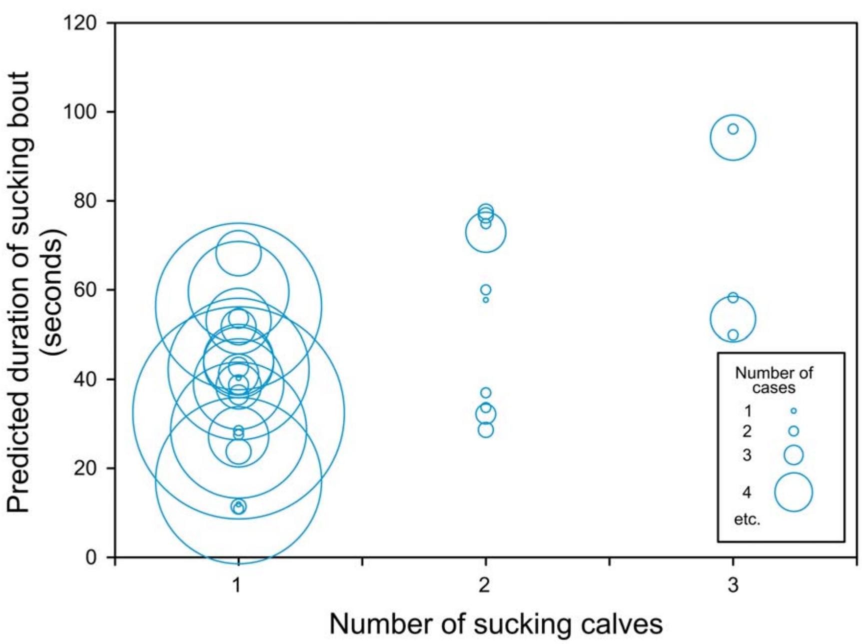

Figure 4. Sucking bout duration depended on number of calves.
doi:10.1371/journal.pone.0053052.g004

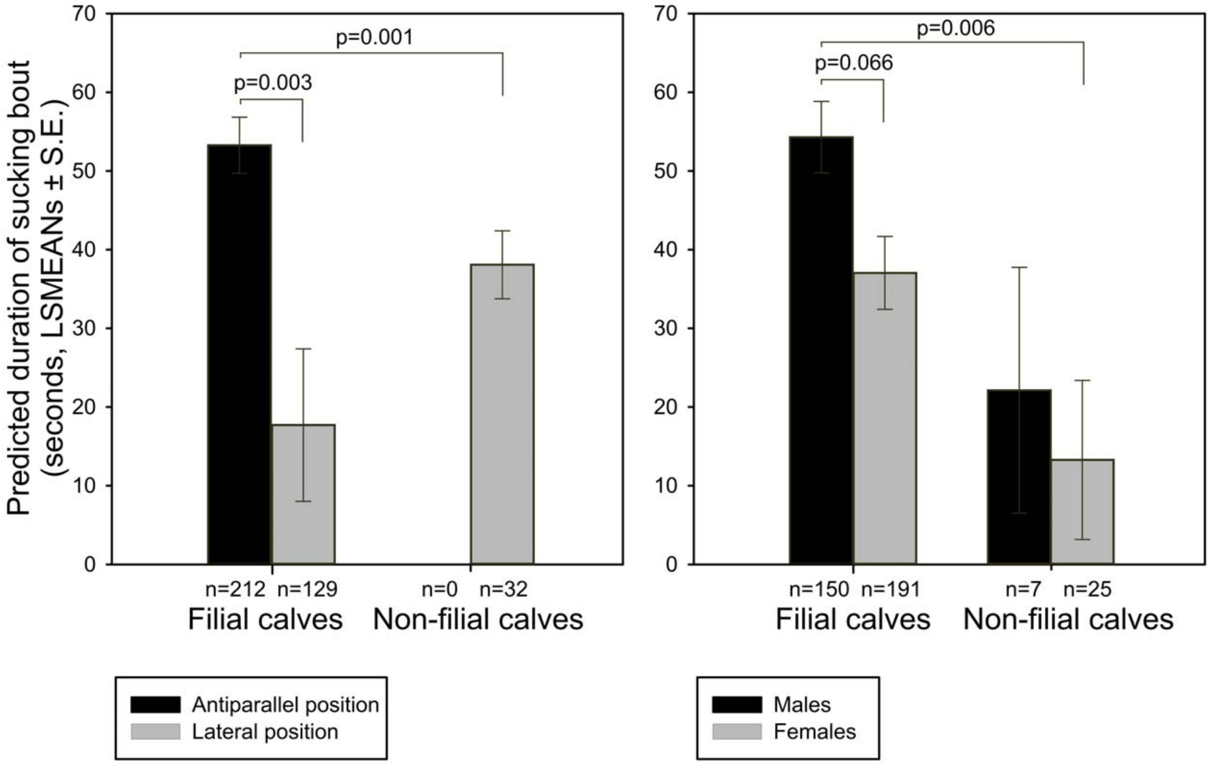

Figure 5. Sucking bout duration (LSMEAN ± SE) for filial and non-filial calves according to position and for filial and non-filial male and female calves.
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than one calf in different zoos and different seasons. The allosucking calves were in all cases the older ones in the herd, while the youngest calf from the herd never allosucked. The only herd in the study without allosucking occurrence was the Brno Zoo in 2006, where two female calves from different mothers were kept together. Although the data were not included in the study, one author (Karolína Brandlová) observed the allosuckling occurrence there out of the range of the recording time (Gaja allosucked from Isis). These data further imply that allosuckling is common in the captive camels, comparable to captive guanaco [20], red deer [4,9], cattle [10], and captive fallow deer [11,12].

Up to three calves (always one filial and one or two non-filials, there were no more calves in the herd than three) were involved together in a sucking bout. The herd with the highest incidence of allonursing (25%) was the only herd with 3 calves providing the largest number of allonursing possibilities. The earliest allosucking was reported in 50-days-old calf. The youngest calves in the herd were never seen allosucking, despite of the fact that they had the possibility to do it just after joining the herd where other nursing female was present. In other ungulates, except in zebra [33], allosuckling was reported from the first day [4] or the first weeks [11,20] of the calves' lives. Even calves with the large percentage extent of allosuckling (up to 100%, Table 2) allosucked only occasionally, being weaned by their mothers and using the opportunity to get surplus milk. Generally, the sucking bouts in this study were on average much shorter than those reported by Sambraus in dromedaries [30] (43 sec and 210 sec, respectively). We found tendency for longer duration of sucking bout for filial males than for filial females (similar to Paranhos da Costa *et al.* [13]). This may be caused by biased investment of females in good condition (with unlimited food supply in captivity) towards male offspring, as shown by Trivers & Willard [34] or simply by higher energetic demands by the larger sex. We did not find this difference for allosuckling bouts.

Evidence for milk-theft hypothesis

Regarding the behaviour of calves, our results widely correspond with the milk-theft hypothesis. We confirmed that (i) allosucking calves sucked only in the lateral (other than antiparallel) position. That may have helped the calf to remain undetected by the nursing female or decreased the probability of being threatened by her. Higher incidence of allosuckling in lateral position was confirmed also by Zapata *et al.* [20] in guanacos. In contrast, the filial calves sucked mostly in the antiparallel position. There was no indication for changing the antiparallel to lateral positions with increasing age.

As predicted (ii), in all cases when more calves were sucking together, the non-filial calves joined filial calf during sucking non-maternal dam and the probability of allosuckling was higher when there were more calves involved in a sucking bout as reported by Ekvall [11], Zapata *et al.*[20] and Pluháček *et al.* [7]. In connection with the lateral position this reflects the obvious tactic not to be seen or threatened by the non-maternal nursing female, characteristic for the parasitisation for the surplus milk described by Packer *et al.* [1].

We failed to find any support for the prediction (iii). We did not record any case of non-filial calf sucking in anti-parallel position, so we could not assess any influence of sucking position on the termination by females. This could mean that calves which tried to allosuck close to the females head were not successful. Females might have refused to nurse them and calves then learned how to approach the non-maternal dam safely and successfully as reported by Zapata [20].

In agreement with our prediction (iv), the incidence of allosucking increased with age of the allosucking calf as skills of the calf to outwit the non-maternal dam increased or as increased the motivation of a calf due to the weaning process of weaning. At least one of the allosucking calves was already weaned. It corresponds with the findings of Ekvall [11] and Landette-Castillejos *et al.* [35], where the allosuckling occurrence increased with the length of lactation.

The suckling bouts generally lasted longer in filial calves in antiparallel position than in non-filial ones in lateral position as we expected (v). Although the sucking duration itself should not be used as a predictor of milk intake, it can reveal the level of maternal investment [36]. The sucking duration for non-filial calves which sucked alone was considerably shorter than in case when they joined already sucking filial calf. Sucking duration was longer for sucking bouts involving more than one calf. This may simply reflect the fact that in longer sucking bouts performed by a filial calf the non-filial calf got greater possibility to notice that the female is nursing, moved close to her and joined the sucking calf. The differences in sucking bout durations are also consistent with milk-theft hypothesis [1].

Evidence for altruistic behaviour

On five occasions a non-filial calf was allosucking with no other calf present (ii). This could be simply a mistake from the dam, considering the fact that mentioned allosucking bouts were considerably shorter than those including also the filial calf. On the other hand, however, we cannot reject entirely the possibility that in some cases the dams tolerated certain individuals in need as an altruistic act as was reported for red deer [4,9] and cattle [10]. Even when the non-filial calf sucked in the lateral position and together with filial calf, we cannot rule out the possibility that the females were able to recognize that they were nursing more than one calf at a time, because the size of allosuckling calves did not allow them to be completely hidden from the sight of the female even in the lateral position.

Termination of sucking bouts by the females did not differ during sucking events involving filial and non-filial calves. We could not test termination of a sucking bout involving a non-filial calf in an antiparallel position (iii) in comparison with a filial calf in the same position, because none of the non-filial calves has ever been seen in the antiparallel position. Taking into account that non-filial calves were sucking more often in the presence of the filial calf and that the non-filial calf was located more distant to the head of the dam, one could presume that the female would terminate equally sucking of filial and non-filial calves when trying to terminate the non-filial sucking. This may explain generally low level of terminations of non-filial sucking bouts and the tolerance of females.

The increasing incidence of allosucking in older calves (iv) may also imply higher tolerance of nursing females to calves that are more familiar to them as they had lived longer in the same herd than the newborn calves.

The fact that some dams allonursed while others did not, and the fact that at least some of the calves sucked regularly and very successfully suggests a possible strategy of compensation of nutritional requirements by the young as seen in red deer and cattle [9,10]. Our data was not adequate for testing this possibility, however. On the other hand, body condition of females did not affect the probability of nursing non-filial calves in guanaco [37].

Age differences among calves in herds were larger in the zoos in this study (the first calf born in January while the last in July, see Tab. 2) than expected in the wild or in semi-captive conditions (several weeks in spring) [25]. This difference may be due to the prolonged breeding season in Europe, which may also increase the possibilities for allosuckling. Similar to Murphey *et al.* [38] and Cassinello [39] at the moment, we may exclude the kinship selection, as the females in the herd were not closely related to one another.

Our results correspond with those of Zapata *et al.* [20,21] for captive and wild guanacos, where the 'milk theft' is most likely explanation of allosucking. As both camels and llamas are adapted to the extreme conditions, the allosuckling occurrence in captive animals could have two possible explanations. First, as camel females live probably in the kin groups [40], allosuckling could have developed as an adaptation for the harsh climatic conditions and can work on the principles of kin selection [1,4,9,12] which should be the objective of further testing proposed also by Zapata *et al.* [20]. Second, females which are kept in less extreme conditions in captivity should have lost the care about what calf is sucking them, and the calves would exploit those possibilities. Moreover, the milk production of captive domesticated camels could be higher than the normal consumption of the calf because of the domestication changes and *ad libitum* food intake in females. Then the females may suffer from the milk overproduction, corresponding with the milk evacuation hypothesis postulated by Roulin [2].

Conclusions

The results of the study support the hypothesis of 'milk theft', being mostly performed by calves behaving as opportunistic parasites. Nevertheless, tolerance of the camel females to non-filial calves may also suggest that at least in part allosuckling in camels might be adaptive trait, despite the fact it is mostly performed by calves which have the occasion to get surplus milk from a non-maternal female as opportunistic parasites.

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Author Contributions

Conceived and designed the experiments: KB LB. Performed the experiments: KB. Analyzed the data: KB LB TH. Contributed reagents/materials/analysis tools: KB. Wrote the paper: KB LB TH.

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5.4. Umělý odchov velblouda dvouhrbého (*Camelus bactrianus*) – Hand-rearing of Bactrian camel (*Camelus bactrianus*)

Figure 8: Artificially reared camel calf was very tame.

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Umělý odchov velblouda dvouhrbého (*Camelus bactrianus*)

Hand-rearing of Bactrian camel (*Camelus bactrianus*)

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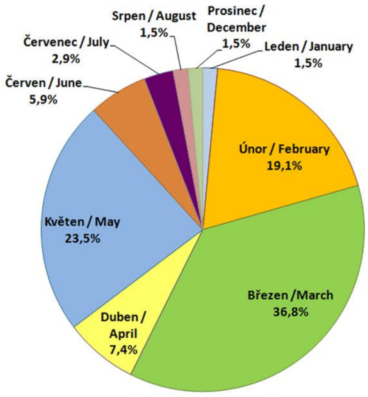
Úvod

Zoo Praha chová velbloudy dvouhrbé (*Camelus bactrianus)* od roku 1932 a přes 50 let úspěšně odchovává průměrně jedno mládě ročně. Nicméně jedna třetina mláďat (31,7 % samců a 40,0 % samic) se z nejrůznějších příčin nedožije prvního roku života (Tab. 1).

Tab. 1 Počty narozených a úspěšně odchovaných mláďat velbloudů dvouhrbých (tedy mláďat, která se dožila svého prvního roku života) v Zoo Praha v letech 1970 - 2011 (M - samec, F - samice)
Tab. 1 The numbers of born and successfully reared calves of Bactrian camels (the calves which survived their first year of the live) in the Prague Zoo between 1970 - 2011 (M - male F - female)

Sledovaný rok Reference year	Narozená mláďata (M,F) Born calves (M,F)	Úspěšně odchovaná mláďata (M,F) Successfully reared calves (M,F)	Sledovaný rok Reference year	Narozená mláďata (M,F) Born calves (M,F)	Úspěšně odchovaná mláďata (M,F) Successfully reared calves (M,F)
1970	0,1	0,1	1991	1,1	1,1
1971	0,2	0,2	1992	1,0	0,0
1972	0,0	0,0	1993	0,0	0,0
1973	2,0	2,0	1994	1,0	0,0
1974	0,0	0,0	1995	1,0	1,0
1975	1,1	1,0	1996	0,1	0,1
1976	0,0	0,0	1997	0,1	0,1
1977	1,1	1,0	1998	2,1	1,1
1978	0,0	0,0	1999	1,0	1,0
1979	2,0	2,0	2000	3,0	3,0
1980	0,0	0,0	2001	1,1	1,0
1981	2,0	1,0	2002	3,0	3,0
1982	1,1	1,0	2003	2,1	1,0
1983	1,1	1,1	2004	0,0	0,0
1984	0,0	0,0	2005	0,0	0,0
1985	2,0	0,0	2006	3,1	1,1
1986	1,3	0,1	2007	1,2	0,1
1987	0,0	0,0	2008	1,0	1,0
1988	3,1	1,1	2009	1,2	1,1
1989	0,0	0,0	2010	1,1	1,1
1990	1,1	1,0	2011	1,1	1,1
			Celkem	**41,25**	**28,15**

Od roku 1970 se narodilo v Zoo Praha 66 mláďat velbloudů dvouhrbých (z toho 62,1 % byli samci). Úspěšně se odchovalo pouze 43 mláďat (65,2 %). Většina mláďat (89,4 %) se v pražské zoo narodila v období od února do května (graf 1). Během tohoto období byly také zaznamenány tři potraty (u dvou samic).



Graf 1 Podíly narozených mláďat v Zoo Praha v jednotlivých měsících (údaje z let 1970 -2011)

Graph1 Proportion of the new born youngs of camels in Prague Zoo in single months (data from the years 1970-2011).

V České republice chová velbloudy většina zoologických zahrad a zooparků. V mnohých z nich se chovatelé v minulosti setkali s komplikovanými odchovy. Některé samice své mládě po porodu odmítají, ovšem při dostatku trpělivosti mládě nakonec většinou přijmou. Občas je ale nezbytné přikročit k umělému odchovu, ten však bývá velmi náročný a často je provázen nejrůznějšími obtížemi jak v období mléčné výživy, tak i v dospělosti zvířat během dalšího chovu. Proto se k umělému odchovu velbloudů nepřistupuje příliš často. Základní doporučení ke krmení osiřelých mláďat podává Coventry (2002). O umělých odchovech velbloudů dvouhrbých v Zoo Brno psal Walter (2007).

Samice Andy (narozena roku 1993) úspěšně odchovala čtyři mláďata. Po úhynu původního samce přišel roku 2003 nový chovný samec Jepe, kterému v té době byl jeden rok, a o tři roky později se začala rodit jeho mláďata. Samice Andy třikrát zabřezla i bez komplikací porodila, ale odchovat se jí nepodařilo ani jedno z těchto mláďat. Hlavním důvodem neúspěchu bylo nadměrné nalití struků po porodu a mládě je nebylo schopno takto veliké vzít do tlamy a úspěšně sát mléko. Masivní otok struků samice spolu s nedostatečným odbytem mléka nakonec vždy vedl k zánětu vemene a jeho bolestivosti, což celou situaci ještě komplikovalo. Mláďata této samice přes veškerou snahu chovatelů (od podstavování pod matku až po oddojování nervózní samice) umírala do pěti dnů od narození.

Umělý odchov v Zoo Praha

23. srpna 2010 samice Andy opět porodila a veškeré problémy se znovu opakovaly. Jelikož mládě vypadalo poměrně čilé a byla to na pohled pěkná samička, tak jsme se rozhodli hned první den od porodu přistoupit k umělému odchovu. Mlezivo dorazilo do zahrady a bylo podáno mláděti druhý den ráno. Do té doby byla samička se svou matkou a všichni doufali, že se alespoň trochu od ní napila. Pokusy napojit mládě za přítomnosti samice byly neúspěšné a riskantní, takže nakonec musela být matka od mláděte oddělena natrvalo. Přesto měla možnost vizuálního i fyzického kontaktu s mládětem přes plot. Po oddělení samice jsme zjistili, že mládě není až tak v dobré kondici, jak to na první pohled vypadalo. Zejména oblast kolem pánve a zadních končetin byla poměrně málo osvalena. Navíc jsme u mláděte poměrně často pozorovali přišlapávání a zamotávání zadních nohou.

Mláděti bylo od druhého dne věku krmeno čerstvé kravské mlezivo, to se ale velmi rychle kazilo, a proto jsme přešli další den na mlezivo mražené. Mlezivo bylo pomalu rozmrazováno a dávka ke krmení se ohřívala ve vodní lázni na 38 °C. Samička byla krmena sedmkrát denně v intervalu po třech hodinách. První krmení probíhalo v 6 hodin ráno a poslední o půlnoci. U mláděte se pravidelně střídali celkem tři chovatelé – Petr Fedorov, Radovan Mourek a Jana Vašáková. Zdravotní stav a aktivita mláděte byly průběžně monitorovány. Kromě množství vypitého mléka a doby, za kterou mládě svou dávku přijalo, bylo zaznamenáváno i jeho močení, kálení a celková čilost mláděte. Mléko bylo podáváno z plastové kojenecké lahve o objemu 250 ml s použitím dětské silikonové savičky s křížovým otvorem. Mládě nemělo problém s takovýmto krmením. Během krmení byl mláděti masírován konečník vlhkým hadříkem, stimulace přispívala k pravidelné defekaci.

Od 5. dne bylo krmení postupně převedeno během jednoho dne z mleziva na mléčnou krmnou směs pro telata Sanolac Rot. Také se mléko začalo podávat ve větší, dvoulitrové plastové lahvi (mléčná lahev pro telata). Podrobný popis frekvence krmení a množství připravovaného mléka je uveden v tabulce č. 2.

Tab. 2 Přehled krmení
Tab. 2 Feeding overview

Věk mláděte (ve dnech) Age of calf (in days)	Typ krmení Type of feed	Počet krmení / den No. of feeding / day	Množství na jedno krmení Amount to one feeding	Poznámky Notes
1 – 4	mlezivo	7x	500 ml	
5	mlezivo + MKS Sanolac Rot	7x	500 ml	
6 – 10	MKS Sanolac Rot	7x	750 ml	
11 – 17	MKS Sanolac Rot	7x	1000 ml	
18 – 20	dle receptu č. 1	7x	1000 ml	
21 – 23	dle receptu č. 1	6x	1000 ml	
24 – 31	dle receptu č. 1	5x	1000 ml	
32 – 50	dle receptu č. 2	5x	1250 ml	
51 – 91	dle receptu č. 3	5x	1500 ml	
92 – 106	dle receptu č. 3	4x	1500 ml	přižírá mačkaný oves
107 – 147	dle receptu č. 3	3x	1500 ml	přižírá více i seno
148 – 171	dle receptu č. 3	2x	1500 ml	
172 – 198	dle receptu č. 3	1x	1500-1800 ml	

Po dvou týdnech odchovu se u mláděte objevil kožní problém. Mládě se neustále drbalo a okusovalo. V této době došlo i poklesu příjmu mléka z průměrných 5 litrů za den na téměř polovinu (graf 2). Velblouděti byl injekčně podán vápník, selen a vitamíny a jednou denně byl po dobu 4 dnů přidáván do mléka přípravek na podporu metabolismu (Vigosine).

Po srovnání složení mléčné krmné směsi pro telata a obsahu živin v kvalitním velbloudím mléce dle dostupné literatury (Iqbal et al. 2001; Konuspayeva et al. 2009) a vzhledem k celkově horší kondici mláděte jsme se rozhodli od 18. dne věku mláděte upravit složení podávaného krmení. Pro zvýšení obsahu živin (zejména tuku, bílkovin a glukózy) začala být k mléčné směsi pro telata přidávána trvanlivá plnotučná smetana ke šlehání (s obsahem tuku kolem 30 %) a Glukopur (hroznový cukr). Použití šlehačky se osvědčilo již v minulosti při umělém odchovu velblouda a podobnou kombinaci doporučuje i Coventry (2002). Během celého odchovu byl přidáván do jednoho krmení denně vitamín C. Směs (viz recept č. 1) byla pečlivě promíchána metlou a přelita přes síto do lahve na krmení. Takto namíchaný nápoj měl teplotu mezi 38–40 °C a bylo možné ho ihned krmit.

Obr. 1 Krmení mláděte v 1. týdnu života
Fig. 1 Feeding of the young in 1st week of its life
Foto/Photo by J. Sebíň

Recept č. 1 (1 litr mléčného nápoje):
400 ml studené převařené vody, 100 ml smetany, 500 ml vroucí vody, 125 g sušené mléčné směsi Sanolac, polévková lžíce Glukopuru.

Přes veškerou snahu se množství přijatého mléka stále snižovalo a 22. den odchovu dosáhlo minima 1140 ml mléka za den. Malá velbloudice byla tou dobou již velmi zesláblá a přestávali jsme věřit, že se odchov podaří. K večeru odmítla pít úplně. Ráno došlo ale k nečekanému obratu a samička na první krmení vypila celý litr mléka. Od té doby se denní příjmy opět začaly zvyšovat.

Od jednoho měsíce věku samička viditelně zesílila, nabírala svalovou hmotu a byla velmi čilá. Touto dobou také přestaly dosavadní zdravotní komplikace a samička vypila za den vždy více než 5 litrů mléka (krmení 5x denně mezi 6. - 22. hodinou dle receptu č. 2 s vynecháním hroznového cukru). O tři týdny později byla zvednuta dávka jednoho krmení (připravováno dle receptu č. 3) na 1,5 litru a celkový denní příjem se pohyboval mezi 6,5-7,5 litry.

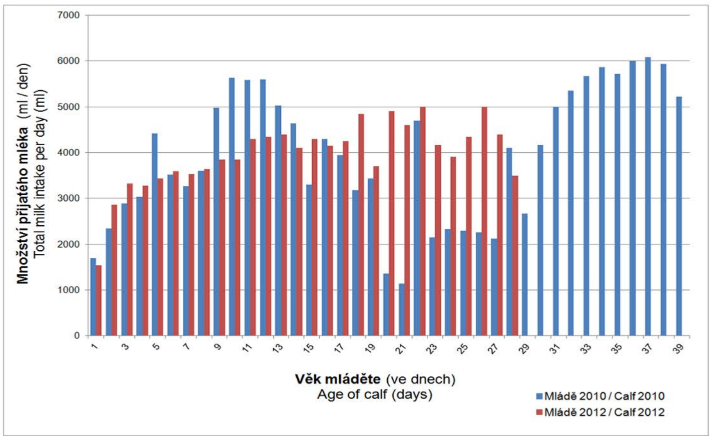
Recept č. 2 (1,25 litru mléčného nápoje):
650 ml studené převařené vody, 100 ml smetany, 500 ml vroucí vody, 150 g sušené mléčné směsi Sanolac.

Recept č. 3 (1,25 litru mléčného nápoje):
650 ml studené převařené vody, 100 ml smetany, 500 ml vroucí vody, 150 g sušené mléčné směsi Sanolac.

Od třetího měsíce také dostala k dispozici vodu k napájení, mačkaný oves a kvalitní seno, aby si začala zvykat i na jiný druh potravy. O toto krmivo ovšem nejevila téměř žádný zájem. Z tohoto důvodu jsme od 4. měsíce snížili frekvenci krmení na 4x denně (mezi 7. – 16. hodinou) a o čtrnáct dní později jen na 3x denně, aby byla motivována vyhledávat i jiný zdroj potravy. Výsledek se dostavil poměrně rychle a příjem pevného krmení se velmi zvýšil. Dále měla možnost během úklidu velkého výběhu, kdy byli ostatní velbloudi ve stáji, chodit na druhý dvorek i do výběhu. Z počátku chodila pouze po vydlážděné části pavlače, na písčitý povrch výběhu se bála vstoupit. Postupem času ale začala chodit i do výběhu.

V průběhu celého odchovu jsme zkoušeli místo silikonové dětské savičky občas použít ke krmení gumový dudlík pro jehňata, který byl stejné velikosti, ale tvrdší. Ten se ale absolutně neosvědčil a samička z něj odmítala pít. Dětské savičky naštěstí poměrně dobře pasovaly i na velkou mléčnou lahev pro telata.

V roce 2012 se opět samici Andy narodilo mládě, které muselo být také uměle odchováno. Tato samička byla v lepší kondici než Narin, což se projevilo zejména na množství přijatého krmiva. Velbloudě během prvního měsíce života pilo bez větších výkyvů, než jak tomu bylo u Narin (viz graf 2). Data z tohoto odchovu bylo možné zahrnout pouze do 1. 4. 2012.



Graf 2 Srovnání množství přijatého mléka v prvních týdnech života u uměle odchovaných mláďat z roku 2010 (Narin) a z roku 2012 (záznamy pouze do 28. dne života)
Graph 2 Comaparation of amounts of received milk in first week of life in hand rearing youngs in the year 2010 (Narin) and from the year 2010 (records only to the 28th day of life of the young)

Zapojení do stáda

Od 4,5 měsíce věku se začala Narin, tak byla samička pojmenována, blíže seznamovat s ostatními velbloudy. Z počátku byla spojována téměř každý den na 2-3 hodiny se samicemi a mláďaty. Samec zůstával po tuto dobu ve stáji, jelikož se jednalo o období říje. Spojování probíhalo většinou v klidu, ale Narin se ostatních velbloudů spíše stranila. Velbloudi si Narin v postatě nevšímali, pouze nejstarší mládě, téměř roční sameček, jí vždy prvních 10 minut

Obr. 2 Krmení mláděte v šesti měsících věku
Fig. 2 Feeding of the young life six months of its life Foto/Photo by J. Sebíň

honil, ale pak se situace uklidnila. Postupně byla nechávána ve výběhu delší dobu a se všemi velbloudy, pouze na noc byla oddělena na svůj dvorek. V té době byla poměrně veliká, hrby měla krásně postavené, plné tukové tkáně a mléko dostávala pouze jednou denně.

Od března, kdy se u chovného samce začala uklidňovat říje, byla vypuštěna do stáda natrvalo. Samec ji občas prohnal po výběhu, ale celkově byla situace klidná. Ovšem 5. den od vypuštění se připletla ve stáji samci pod nohy a ten ji kousnul do ramene. Narin měla vytržené chomáčky chlupů, ale nejvíce utrpěla asi psychicky. Od té doby se začala bát všech velbloudů. Proto byl velbloudům omezen přístup do stáje a ve velkém výběhu samička neměla problém se komukoliv ze stáda vyhnout. Seno bylo podáváno na dvě krmná místa, aby měla možnost se vždy v klidu nažrat. Postupně se opět začala seznamovat s velbloudy a nejvíce času trávila s mladým samečkem, který byl čtvrt roku starší než ona. Čím dál tím častěji se přidávala u krmení také k samicím. Přesto trávila poměrně dost času samostatně.

Tři měsíce od vypuštění do stáda již nebylo poznat, že by Narin byla uměle odchována. Žrala se všemi členy stáda (někdy i samotná se samcem) a chodívala si k nim i lehat. Ovšem stres ze samce a spolu s důsledky časného odstavu se podepsaly na její kondici. Hrby jí opět polehly a přesto, že všichni velbloudi měli v červnu již letní srst, malá samička měla stále zimní srst. Kvůli zhoršené kondici se Narin začala v létě oddělovat na zvláštní příděly koncentrovaných krmiv. Na podzim došlo k úhynu nejstarší velbloudice, po které zbylo půlroční mládě. To sice chodilo pít i k jiné velbloudici, ale stále jsme se obávali nedostatečného příjmu živin u těchto dvou velblouďat. Proto jsme zrealizovali plán přikrmování mláďat. Mezi výběh a oddělovací dvorek byla do vchodu namontována ve výšce 160 cm snímatelná kovová příčka. Mláďata tak mohla volně procházet na dvorek, kde bylo podáváno doplňkové krmivo (granulát a oves), aniž by se krmení zmocnila dospělá zvířata, která se pod příčku nevešla díky hrbům. Kondice obou mláďat se začala od té doby velmi zlepšovat.

Diskuze a závěrečná doporučení

Při odchovu je velmi důležité podat mlezivo v co nejkratším možné době od narození, nejlépe oddojené přímo od matky (Čermák 2007). Narin dostala pravděpodobně své první mlezivo až 24 hodin po narození, a to kravské a původem z naprosto cizího prostředí. Všechny

Obr. 3 Příčka ve výšce 160 cm umožní průchod ke krmení pouze mláďatům. Největší mládě je uměle odchovaná samička Narin
Fig. 3 The barin 160 cm level does allow the passage to feeding places for youngs only. The biggest young one is the hand reared female Narin Foto/Photo by T. Haberová Foto/Photo by T. Haberová

tyto faktory mohly mít na následek snížení imunity mláděte a její zdravotní problémy v průběhu prvního měsíce života.

Pokud není možné pravidelně (alespoň dvakrát denně) získat čerstvé mlezivo, doporučujeme používat mražené, u kterého nehrozí zkvašení. Podle dosavadních znalostí zmražení mleziva nemá výrazný vliv na množství protilátek v něm obsažených, pokud je mlezivo rozmrazováno pomalu (Holloway et al. 2001, Čermák 2007). Přechod z mleziva na mléčnou náhražku by měl být rozdělen alespoň do dvou dnů, tím lze snížit riziko zácpy u mláďat.

Na krmení mláděte by se mělo podílet co nejméně lidí. Mládě i chovatelé si musí vytvořit určitou praxi a rutinu při krmení. Střídání více lidí na začátku odchovu zmenšuje množství přijatého krmiva.

U odchovu velbloudů je vhodné mléčné krmné směsi pro telata doplnit šlehačkou, hroznovým cukrem a vitamínem C, aby složení mléka lépe odpovídalo potřebám mláděte. Pokud osiří velbloudě, které je zvyklé pít mléko od samice, tak je dobré zvážit možnost kojení jinou samicí v laktaci. U velbloudů chovaných v lidské péči se tzv. allokojení vyskytuje poměrně často a mnohé samice nechají sát mléko i cizí mláďata (Haberová & Koláčková 2010). V tomto případě je ale třeba zvážit i aktuální kondici kojící velbloudice a mláďata by měla být přikrmována koncentrovanými krmivy.

Načasování hraje velkou roli v managementu odchovu. Narin se narodila v srpnu, proto její spojení se stádem připadlo na tu nejnevhodnější dobu – vrchol zimy a tedy období říje samce. Pokud by se narodila na jaře, jak tomu obyčejně bývá, tak by spojení se stádem proběhlo v průběhu léta, tedy v době kdy odrostlá mláďata již nebývají ve stádě a kdy je samec naprosto klidný. Takto se mohlo předejít zhoršení kondice mláděte v důsledku stresu. Doba spojování se skupinou velbloudů a odstavu by se neměli překrývat. Mládě je vhodné spojit se stádem v co nejnižším věku, pokud to situace dovoluje, a nadále ho přikrmovat ve stádě.

Ačkoliv Coventry (2002) udává délku mléčné výživy velblouďat 5 měsíců, je lépe úplný odstav mláděte prodloužit na 9 měsíců věku. Tím se zajistí optimální kondice odchovaných zvířat. Toto je logické i vzhledem k faktu, že velbloudice kojí mláďata až 18 měsíců (Jasra et al. 1999). Nicméně časný odstav je u velbloudů také možný, pokud je mláďatům poskytnuto dostatečné množství živin (Degen et al. 1987).

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SUMMARY

Camels are bred by most zoological gardens in the Czech Republic and many of them had some problems with rearing of calves in the past. But the artificial rearing is very time-consuming and commonly accompanied with problems. That is why the artificial rearing of camel calves is not common in the zoos. Some basic information about feeding of orphan camel is given by Coventry (2002).

Prague zoological garden has bred Bactrian camels (*Camelus bactrianus*) from 30th years of the 20th century but only 65.2 % of calves were successfully reared. Table 1 gives the information about number of births and number of reared camel calves in particular years. The most births took a place from February to May (see Graph 1).

The cow Andy (born in 1993) reared four calves successfully but in 2003 she started to have problems with the teats and now she is not able to nurse her calves. This cow gave another birth on the 23rd August 2010 again and we decided to rear her calf artificially.

The first colostrum was fed to the calf next morning (second day of its life). We fed her fresh colostrum from domestic cattle and later frozen colostrum which had a better durability. The drink was fed with the temperature about 38° C seven times between 6 a.m. and midnight (every three hours). We noted the amount of accepted feed, the length of feeding, the activity of the calf, its healthy status, and the urination and defecation.

The colostrum was fed from the plastic baby bottle (250 ml) with the silicon teat for children. The camel cow had to be separated from her calf for our safety but they had a contact through the fence.

The colostrum was replaced by the milk replacer for cattle (Sanolac Rot) during one day in the fifth day of the calf's age. The milk replacer was fed from the bigger plastic bottle for calves. We used the silicon teat for children all the time because all attempts to replace it with the rubber teat for lambs failed.

We improved the composition of the milk replacer by the addition of skim (fat content about 30 %) and glucose. This composition was more similar to camel milk (Iqbal et al. 2001, Coventry 2002, Konuspayeva et al. 2009). We also added vitamin C to the milk once a day. The complete overview of the feeding is written in table 1 and the milk recipes are below.

Recipe 1 (1 litre of milk replacer):
400 ml of cold water, 100 ml of skim, 500 ml of boiled water, 125 g milk replacer powder Sanolac, tea spoon of glucose.

Recipe 2 (1.25 litre of milk replacer):
650 ml of cold water, 100 ml of skim, 500 ml of boiled water, 150 g milk replacer powder Sanolac.

Recipe 3 (1.5 litre of milk replacer):
600 ml of cold water, 150 ml of skim, 700 ml of boiled water, 190 g milk replacer powder Sanolac.

We noted the big decrease of accepted milk from the third week of the calf's age. This problem was also connected with skin infection. The calf became very weak and we expected that it would not survive but the situation turned and the calf started to feed better again (see Graph 2). The calf become stronger from the first month of age and its condition was much better as well.

We offered the calf water, oats and meadow hay from the third month of age to habituate it to a solid feed but the calf did not prove any interest in it. We restricted the milk feed to four and later three times a day one month later. This step led to the increased income of a solid feed.

The calf was allowed to walk to the run while the herd was closed in the stable. Later it was also introduced to the camel cows and other calves without any problem. The camel bull was in this time in the heat. By the time, the calf habituated to stay in the run with the whole herd and during the whole day. But unfortunately, the bull bit the calf and from this moment the calf feared all camels. Its fright disappeared slowly but this stress connected with the weaning led to bad body condition of the calf. We started the supplementary feeding of all calves on the yard separated by a metal bar in the height of 160 cm. Adult animals could not walk through this bar and so all feed was eaten by the calves. The body condition of the calves soon got better.

The camel cow Andy gave a birth again in 2012 and her calf was also reared artificially. This newborn female calf was in better condition than the previous one and it ate better as well (see Graph 2).

6. DISCUSSION

This PhD thesis dealt with uncommon topics focusing on camels. The author published for the first time the application of the Cuboni reaction and BaCl test in urine and salivary hormonal assay as non-invasive methods of pregnancy diagnosis in camels. Hormonal changes in camels kept in Europe had never been studied before. The research into these techniques emerged from a lack of methods which could be applicable in practise with camels, being such extraordinary animals. Next, we described the maternal behaviour and occurrence of allosuckling in camels kept in zoological gardens, which was also the first, and we share our experiences with the artificial rearing of camel calves.

As a result, the Cuboni reaction can be used for pregnancy diagnosis in camels. Although this test is applicable in the later stages of pregnancy and cannot reach the same accuracy as pregnancy diagnosis by rectal palpation (Vyas and Sahani, 2000), transrectal ultrasonography (Skidmore et al., 2000; Khatir and Anouassi, 2006) or blood progesterone assay (Bakheit et al., 2008), the benefits of the Cuboni reaction are based on non-invasive sampling which can be done by the keeper without the assistance of other personnel, and the inexpensiveness and simplicity for the keeper, who only collects samples and delivers them to the State Veterinary Institute in the Czech Republic. The Cuboni reaction is also offered by diagnostic laboratories in other countries such as the United Kingdom (e.g. http://ctdslab.co.uk/ or www.idexx.co.uk) or Belgium (e.g. www.vetbiolab.be). Of the three tested non-invasive methods, the Cuboni reaction seems to be the best for application in practise.

In contrast, the barium chloride test did not give convincing results. The results of the test were significantly affected by the colour (Pearson chi-sq.: 14.9215, df = 2, p = 0.001) and specific gravity of the urine (Pearson chi-sq.: 8.152958, df = 2, p = 0.0170). Moreover, the results gave a reversed trend compared to other studies (Ndu et al., 2000; Lalrintluanga and Dutta, 2009; Rao and Veena, 2009). Camels are known for their ability to produce more concentrated urine as an adaptation to dehydration (Kataria et al., 2004; Kataria et al., 2007) and the mean specific gravity of swine (Krogh et al., 1979; Almond and Howell, 1993; Coma et al., 1995) and domestic cattle (Weeth and Lesperance, 1965; Beatty et al., 2006) is lower than in camels (Read, 1925; Banerjee et al., 1981; Amer and Alhendi, 1996) and unpublished result of PhD thesis author), which may be a possible cause of the different results. Significant differences between the results of the Cuboni reaction and the barium chloride test were found (Wilks' lambda = 0.33592, F (6, 40) = 4.8358, p = 0.00085).

Despite the fact that analyses of salivary sex steroid hormones are not as easy, and its application by keepers in zoological garden is not expected in practise, we showed that it is possible to collect saliva from camels non-invasively and hormonal assays are feasible. A disadvantage of this method is the fact that the concentrations of hormones in saliva fluctuate (Hofman, 2001; Kobelt et al., 2003) and no laboratory offers these assays routinely in the Czech Republic. We were not able to test if this method is usable for heat diagnosis, because it was not possible to detect heat precisely. But we determined significant changes in progesterone during the year. For the future, it would be beneficial to compare hormonal concentrations in saliva with concentration in another body fluids like blood or urine. A positive correlation between salivary and serum oestradiol was already reported by Qureshi et al. (1999) in buffaloes and the fact that serum hormonal concentrations correlated with concentrations in saliva was well proved in human (Hofman, 2001). Unfortunately, regular blood sampling in half-tamed camels kept in zoo is impossible.

As it was already proved by our study (Fedorova et al., 2015), sampling of urine in half-tamed camels is feasible, however sampling can sometimes be time consuming. According to author's experiences, the researcher can wait for urination of camel more than five hours in some cases and there is always risk of animal's frightening which could lead to interruption of urination. Non-dehydrated camels urinates seven to eight times per 24 hours (Khan et al., 1998) and the production of urine is reduced during dehydration (Ben Goumi et al., 1993) so sampling can last quite a long time, especially in tropics. Moreover, camels urinates more often during a night (Bhakat and Chaturvedi, 2004). From this reasons, routinely and intensive non-invasive sampling of urine is not very useful in practise. In comparison, sampling of saliva lasted only few minutes. In 20 cases, it was possible to compare both the salivary hormonal assay and the results of the Cuboni reaction, however no significant connection between these two methods was found ($p > 0.05$). The small number of cases, together with the fact that hormonal levels in urine are more stable (Monfort, 2003) could cause this incompatible result.

Our finding that the occurrence of allosucking is so common in camel herds confirmed the experiences of camel keepers from zoological gardens. This behaviour negatively affects the body condition of allonursing females, because nursing is very energetically costly (Waltner et al., 1993; Gallo et al., 1996). Moreover, the growth and body condition of camel calves with allonursing mothers can also be negatively influenced. In contrast, a positive effect on the growth of allosucking young ones was not proved (Bartoš et al., 2001). But the growth of calves can be positively affected by management, e.g. by offering supplementary feed to calves using a creep feeder or similar system, as mentioned in the article about artificial rearing of camel calves by Haberová and Fedorov (2012). Nevertheless, the occurrence of allosuckling in herds can also provide some benefits for breeders. Camel females which tolerate the suckling of non-filial calves can serve as nurses for orphaned calves, and these females can provide at least partial milk nutrition to the calf. However, here it is important to know which female tolerates allonursing and if the calf is performing allosuckling; an allosuckling calf will adapt to the loss of its mother better, as reported in Haberová and Fedorov (2012). Paranhos da Costa et al. (2000) gave a similar recommendation.

More detailed research focused on maternal and suckling behaviour in camels is needed. Especially, when occurrence of allosuckling was such high in zoological gardens (Brandlova et al., 2013). The questions why camel females tolerate suckling of non-filial animals and why allosucking developed in camel calves were still not fully answered. The causes of intersucking, which was also observed in camels (Haberová and Koláčková, 2010), are usually connected with milk feeding techniques and management of artificially reared calves of cattle (Keil and Langhans, 2001; Lidfors and Isberg, 2003) however the management of intensively raised cattle and camels reared in zoological gardens is rather different. If the management can play some role in occurrence of allosuckling, the non-invasive pregnancy diagnosis in camels is even more need. The occurrence of allosuckling was not yet reported in camels kept as husbandry animals and so the similar research in tropics would be welcomed.

Two camel calves were successfully reared in Prague zoo. The artificial rearing of camel calves is sometimes practised in zoological gardens or by private breeders when natural rearing is not possible, but no uniform manual exists for the conditions of Central Europe, and the sharing of such information is sometimes difficult. Moreover, artificial rearing is usually carried out without any deeper knowledge of camel milk composition. E.g. the simple addition of vitamin C, which is naturally higher in camel milk compared to domestic cattle (Farah et al., 1992; Zhang et al., 2005), can be beneficial for the health of the calf (Hemingway, 1991; Eicher-Pruiett et al., 1992).

7. CONCLUSIONS

This thesis concludes that non-invasive pregnancy diagnosis in half-tamed captive camels is applicable, allosuckling is a quite common phenomenon in camels bred in zoological gardens, and the artificial rearing of camel calves based on an improved formula of commercial milk replacers for cattle can be successful.

The studies presented in this thesis will be followed by other research and publications. Some of the results obtained from the research have not yet been published, e.g. saliva crystallization in camels, urinalysis of camel urine and a more detailed study concerning the allonursing behaviour of captive camels, which is currently being prepared for publication. Moreover, the author of the thesis built up a team over the last three years which is focusing on non-invasive pregnancy diagnosis in ungulates and non-tamed zoo animals; four MSc theses have already been successfully defended and other studies are ongoing.

Our results are continuously shared and discussed with camel keepers and zoologists from zoological gardens, and we hope that our findings will contribute to the even better management and welfare of camels and other animals kept in zoological gardens and private holdings.

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Selected Conference Papers:

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Appendix 1: Breeding seasons in different countries

Country	Camel species	Breeding season (and its peak)	References
United Arab Emirates	dromedary	October to April (November to January)	Aboulela (1994); Nowshari and Ali (2005); Skidmore and Billah (2006)
Saudi Arabia	dromedary	October to June (November to March)	Abbas et al. (2000)
Oman	dromedary	November to early March	Musa et al. (2000)
Israel	dromedary	January to April	Yagil and Etzion (1980)
Egypt	dromedary	November to April (February to April)	Hussein et al. (2008); Ismail et al. (1998)
Morocco	dromedary	December to April	Sghiri and Driancourt (1999)
Sudan	dromedary	May to October (July to August)[1]	Kohler-Rollefson et al. (1991); Abbas et al. (1992); Musa et al. (2006)
Pakistan	dromedary	December to March	Aujla et al. (1998)
India	dromedary	December to May (January to April)	Deen et al. (2003)
China	Bactrian camel	December to April (January to February)	Zhao et al. (1994)
Australia	dromedary	May to December	Dörges and Heucke (1995); PISC (2006)
Czech Republic	Bactrian camel	November to July (January to April)	Haberová and Fedorov (2012)
Germany	not specified	December to April	Puschmann et al. (2013)

[1] In Sudan two breeding seasons occur; a second (minor) one takes place between December and January

Appendix 2: Composition of camel milk and colostrum

Type of milk	Camel species	Location	Average milk composition (and range)					References
			Dry matter (%)	Protein (%)	Lactose (%)	Fat (%)	Ash (%)	
mature milk	dromedary	Kenya	12.2	3.11	5.24	3.15	0.80	Farah and Ruegg (1989)
mature milk	dromedary	Saudi Arabia	11.7	2.81	4.16	3.15	0.83	Elamin and Wilcox (1992)
mature milk	dromedary	Kazakhstan	-	3.05 (2.58–3.64)	3.46 (2.56–4.84)	6.04 (4.34–7.81)	-	Konuspayeva et al. (2010a)
mature milk	dromedary	Israel	11.5 (10.1–14.1)	2.79 (2.12–3.45)	4.81 (4.43–5.53)	3.39 (2.20–5.30)	0.77 (0.68–0.89)	Guliye et al. (2000)
mature milk	dromedary	Germany (zoo)	-	2.69 (1.87–3.07)	5.40	4.47	-	Puschmann et al. (2013)
mature milk	Bactrian camel	China	14.31	3.55	4.24	5.65	0.87	Zhang et al. (2005)
mature milk	Bactrian camel	Germany (zoo)	-	3.60 (2.82–4.62)	5.16	3.05	-	Puschmann et al. (2013)
colostrum	dromedary	Kazakhstan	-	6.03	3.63	7.88	-	Konuspayeva et al. (2010b)
colostrum	dromedary	Germany (zoo)	-	13.08 / 5.35[2]	4.97	0.22–0.50	-	Puschmann et al. (2013)
colostrum	Bactrian camel	China	20.16	14.23 / 7.17[1]	4.44	0.27 / 4.18[1]	0.77	Zhang et al. (2005)
colostrum	Bactrian camel	Germany (zoo)	-	13.86 / 4.82[2]	4.00 / 5.20	0.22–0.50	-	Puschmann et al. (2013)

[1] Content 2 hours / 48 hours after parturition

[2] Content 1 day / 3 days after parturition