WBC / ICAR 2008 SATELLITE MEETING ON CAMELID REPRODUCTION

12-13 July, 2008, Budapest, Hungary

Program and Extended Abstracts

Peter Nagy; Gyula Huszenicza; Judit Juhasz (Eds.)

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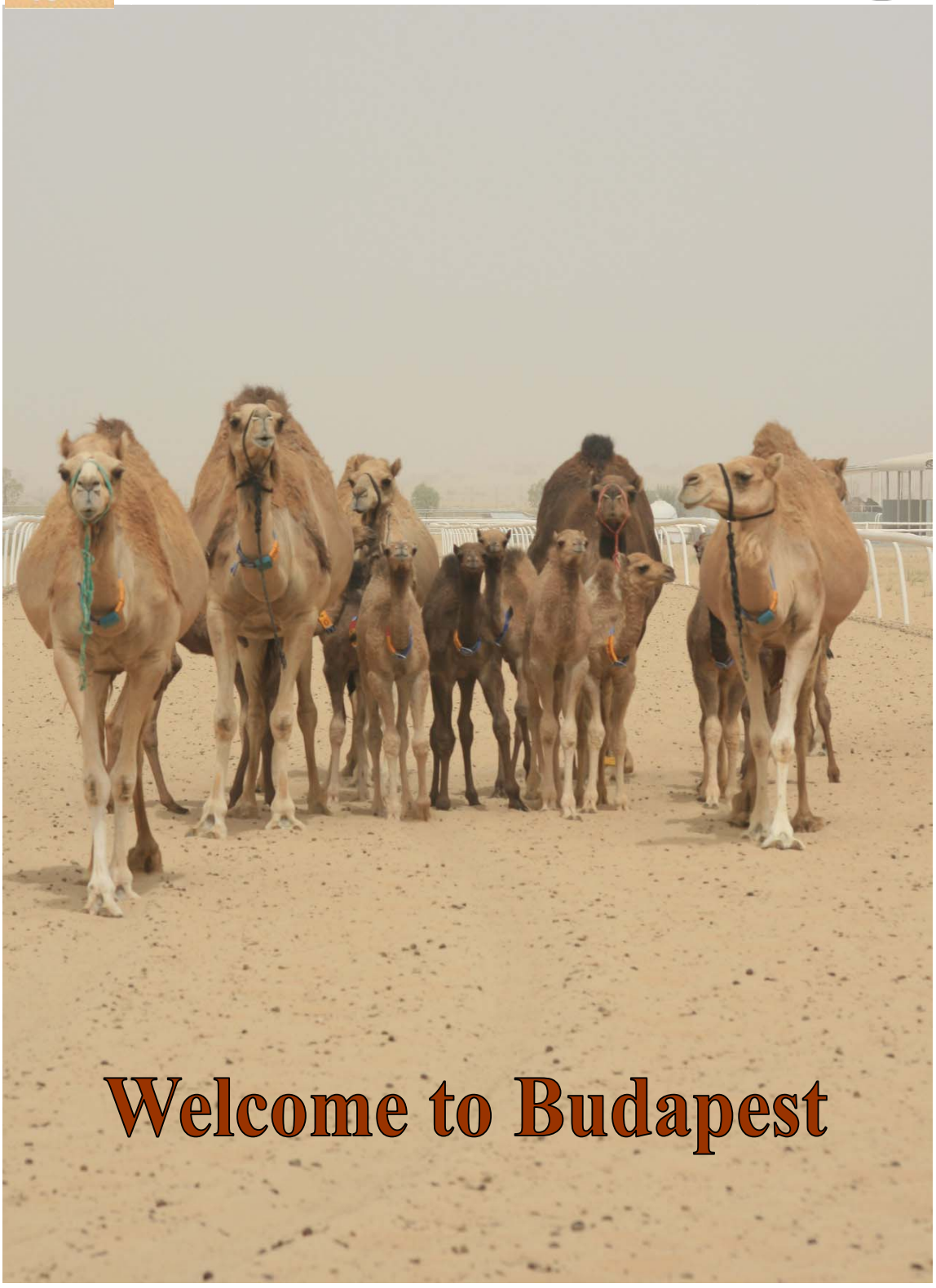

Welcome to Budapest

PROGRAM AND EXTENDED ABSTRACTS

WBC / ICAR 2008 Satellite Meeting on Camelid Reproduction

Organized by

Emirates Industries for Camel Milk & Products (Camelicious™)

Dubai, United Arab Emirates

Faculty of Veterinary Science, Szent Istvan University

Budapest, Hungary

Budapest Zoo & Botanical Garden

Budapest, Hungary

In co-operation with

International Veterinary Information Service (IVIS)

Ithaca, New York, USA

Barlang ("Cave") Hall
Budapest Zoo & Botanical Garden
Budapest, Hungary
July 12 – 13, 2008

WBC / ICAR 2008 Satellite Meeting on Camelid Reproduction

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Program

12 July 2008, Saturday

Registration: 11.30 – 13.00

Opening

13.00 – 13.15 Prof. Miklos Persanyi, Director General, Budapest Zoo
Prof. László Solti, Rector, Szent István University, Gödöllő - Budapest
Prof. László Fodor, Dean, Faculty of Veterinary Science, Budapest

1. Introduction

13.15 – 13.45	Bernard Faye: The production potential and importance of camels and Camelids in the world	1
13.45 – 14.00	Coffee break	

2. Review of Camelid Reproduction
moderators: *Chis Maxwell* and *Gyula Huszenicza*

14.00 – 15.00	Amir Niasari-Naslaji: An update on Bactrian camel reproduction	5
15.00 – 15.15	Coffee break	
15.15 – 16.00	Gregg Adams: Mechanism and control of follicular growth and ovulation in New World Camelids	--
16.00 – 16.45	Lulu Skidmore: Developments in reproduction in dromedary camels	10
16.45 – 17.15	Coffee break	
17.15 – 18.00	Marcelo Miragaya: Advances in reproductive biology in South American Camelids	14
18.15 – 19.30	**Guided tour in the Budapest Zoo & Botanical Garden**	
19.30	Gala dinner (Bagolyvár Restaurant)	

13 July 2008, Sunday

3. Male physiology, semen quality, processing and artificial insemination
moderator: *Marcelo Miragaya* and *Jane Vaughan*

4. Embryo transfer, related techniques and genetics
moderator: *Lulu Skidmore* and *Alex Tinson*

5. Reproductive efficiency and disorders of the reproductive tract
moderator: *Gregg Adams* and *Aminu Deen*

6. Camelids as traditional and/or modern production animals and related diseases
moderator: *Bernard Faye* and *Amir Niasari-Naslaji*

Preface

In a large part of the world, Old and New World Camelids are vital for the livelihood of the local population. Despite this fact, these species usually receive little attention for scientific research and opportunities for direct interaction between professionals are scarce.

This summer, 2 major events, the XXV World Buiatrics Congress and the 16th International Conference on Animal Reproduction have been organized in Budapest during 2 consecutive weeks bringing many professionals and scientists together from all continents. This was a unique opportunity for us to set up a separate satellite meeting focusing only on Camelid Reproduction. Our aim is to promote the development of reproduction research, to draw the attention of the scientific community and international organizations to this field and to facilitate communication between colleagues.

The meeting could not have been organized without the support of various organizations. To organize the conference, we brought together the private industry (EICMP), academic (FVS) and government (Zoo) institutions, and a not-for-profit veterinary organization (IVIS).

I am really delighted that all of our colleagues and friends working on this field, welcomed the idea and most of them will be able to participate at the meeting. I am grateful to those colleagues who were involved in the local organization and in the scientific committee. The program includes reviews and original research papers covering the most important topics of reproduction in different species of Camelids. There will be some lectures with exciting new topics.

The proceedings include extended abstracts (4-5 pages) of presented lectures that will also be published in the IVIS website. We hope that this form of communication will allow the fast distribution of information summarized at the meeting.

We are really looking forward to seeing you in Budapest!

Peter Nagy
Chairman of the meetin

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The production potential and importance of camels and Camelids in the world

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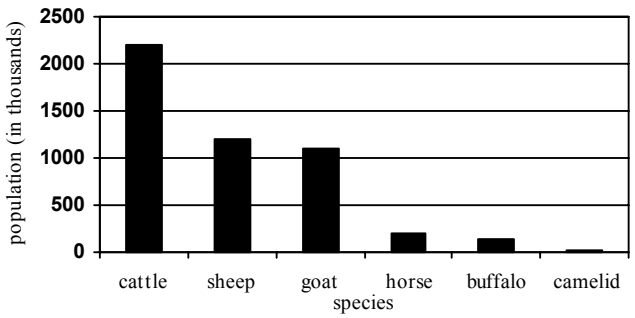
Introduction: For most of the people, camelids are not linked to zootechnical productions. Yet, large and small camelids play an important role in arid lands or high mountains for milk, meat, wool and energy production. The potential of camelids is generally underestimated for two main reasons: (1) the milk and meat production is yet mainly intended for subsistence farming, or, in case of surplus considered as a gift, (2) only few references are available, even if recording data are now more reliable than in the past. For example: a recent exhaustive study on camel milk gross composition (Konuspayeva, 2007) included 82 available references only. Elsewhere, as the camelids are living most of the time in remote areas (desert or high mountains), their accessibility could be difficult. So, performances' controls are scarce or not reliable or limited to very few animals. However, according to some convenient surveys, camelids are essential for animal protein supply of human in these margin areas, contribute to the maintenance of rural activities and economical development, and finally facilitate the integration in the global economy.

The place of the camelids in the animal production: the world camelid population (21 millions large and 8 millions small camelids, probably underestimated) is not important compared to other herbivorous species (fig.1). However, they represent 12% domestic herbivorous biomass in African arid countries and 2% in Asia.

Fig.1: World herbivorous population



Milk: 85% of the milk produced and marketed throughout the world is cow milk. The camel milk production occupies a tiny place (<1%), far behind the buffalo or even the goat and ewe. According to FAO statistics, the camel milk production was around 1,300,000 tons in 2006 that is quite low and probably underestimated. A different statement can be formulated starting from the extrapolation of the yield awaited for a lactating female. According to the proportion of lactating females of 18% (Hjort af Ornäs, 1988), and an average production of 1500 litres per year, the world production can be estimated to 5,7 million tons of which approximately 55% are taken by the calf (Faye, 2004). The individual dairy production is not well documented in the literature data. A range of 1000 to 3300 litres/lactation is reported in different papers from Africa. In Asia, a wider range is reported from 650 to 6000 litres, even more (Khan and Iqbal, 2001). Finally, the dairy potential of camel appeared higher than that of the cow reared under the same climatic and feeding conditions. In Ethiopia, the Afar farmers rearing simultaneously cattle and camel, get on average 1 to 1.5 litres of milk with afar zebu against 4 to 5 liters with Dankali camel (Richard and Gerard, 1985). According to observations' of Schwartz and Dioli (1992) in the Horn of Africa, reported to the live weight of the animal, the dairy productivity of camel (250 kg/TLU/year) is higher than that of the small ruminants (220 kg) and than that of the zebu cattle (100 kg). Genetic variability is reported (Ismaïl et Al-Mutaïri, 1998), but the selection pressure is generally low.

Meat: The camel meat production represents about 0.7% of the world meat production, i.e. 216,315 tons (FAO, 2006), but informations are quite difficult to collect as the main part of the camel meat data comes from the informal market. Traditionally, camel meat consumption is not common in a subsistence system, the size of the carcass needing to share the meat between a wide numbers of people. However, the urbanization has increased the camel meat demand in most of the arid countries. The exploitation rate concerns on average less than 1% of the animals (class 0-2 years), 2.6% (class 2-3 y), 4.7% (class 3-4 y), 13.2% (class 4-5 y) and 15.4% beyond 5 years (Hjort Af Ornäs, 1988) but with a wide geographical variability according to feeding practices and priority given to the milk production. In this case, the sacrifice of the young males could be more important in order to recover the milk normally intended for the calf. In the Horn of Africa, where camel fattening is traditional, the dressing rate can reach 59 %

and the carcass weight, up to 300 kg. Live weight up to 1300 kg has been reported in Arvana breed in Turkmenistan. In South America, lama and Alpaca meat is considered mainly as a by-product of the wool production which is more well-paid by the international market. In Peru, 8000 tons/y of alpaca meat are available, mainly consumed locally. Camels have a low growth rate, late puberty, long gestation time, so, the meat productivity is lower than for other ruminants: 7.5 kg/TLU/year *vs* 14 kg for cattle and 35 kg for small ruminants. In traditional conditions, the Daily Growth Rate (DGR in g/day) for one-year camel is 190 to 310g (Faye et al., 1992). In more intensive conditions, it can reach 440-580g. In Australia, a maximum of 1100g was reported.

Power: The camel is used for packsaddle, draught and race. As a pack animal, it is able to walk at 4-5 km/h for 10 hours with 150 to 300 kg on the back. Extreme values with 400-500 kg are reported in Pakistan. In Niger, the weight of the packsaddle is between 200 and 250 kg. The pack camel could transport this charge for 30 to 35 days, walking 60 km each day (Pacholek et al., 2000). The camel is commonly assigned to agricultural works (ploughing, carting, and sowing). Its performances are similar to horse (Schwartz and Dioli, 1992). The racing camel can run 50 to 100 km per day at the speed of 10-12 km/h. In short race (10 km), the best runners can reach 34 km/h with a maximum of 40 km/h.

Other products: Camelids are appreciated for the high quality of their wool (alpaca and Bactrian camel). Recently, the camel leather industry was better valorized in relationship with the touristic activities (especially in Tunisia).

The news trends in camelid production. Three main trends are observed in the camelid world: (i) **intensification of the camel production** with the development of modern dairy farms with high dairy potential lactating camels (as the Al-Majahim breed from Saudi Arabia) and industrial milk processing (pasteurized milk, cheese making, modern packaging), or camel feed-lot with intensification of the reproduction process; (ii) **diversification of the camel production** with increasing of a wider using of camelids in agriculture activities, carting, leisure (mainly for small camelids), increasing demand of camel milk and meat for dietetic and medicinal purposes, (iii) **increasing of the distribution area** in the world linked to the climatic changes (Faye et al., 2008), to

the diversification of their use, to the aridification of many parts of the world, to the increasing of the camel products demand in urban areas.

Conclusion: The scientific community plays an essential role for considering camel under three aspects underlying the importance of camelids, now and in the future. The camelids are interesting as a biological model, as a productive animal for food supply in remote areas, and as an element of the arid ecosystem where they contribute to the desertification combat and food security. The camel scientists have to convince the funding agencies for the high interest of the camelids for the promotion of desert productivity and preservation. However, some lack in camel researches can be considered (reproduction, genetic, milk and meat processing, emerging diseases, and farming economy). The creation of ISOCARD (International Society of Camelid Research and development) is a quite important step to stimulate coordination research between the different camel sciences network through the world.

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An update on Bactrian camel reproduction

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Introduction

Bactrian camel population in Iran is threaten with extinction; although, it is assumed that this species has been domesticated on the eastern border of the Caspian sea around 2500 B.C. and from there it was migrated to several countries worldwide (8). Present manuscript will summarize our recent findings on controlling follicle wave cycle and applying reproductive technologies in Bactrian camel.

Semen collection and processing

Semen was collected using a modified bovine artificial vagina (1). The inner surface of the AV liner was covered with a thin layer of sterile petroleum jelly. Semen was collected after she-camel was physically restrained in sternal recumbency (7). Viscosity, as the major constraint in the processing of camel semen (1), was reduced using a simple mechanical approach (7).

Semen biophysical and biochemical characteristics

The color of Bactrian camel semen was milky (7). The volume of the ejaculates averaged 8.2±0.7 ml (1.2-26 ml; Table 1; 7). The average osmolality of Bactrian camel semen was 318.2±1.9 mOsm/kg H_2O (300 to 348 mOsm/kg H_2O; Table 1; 7). The pH of the seminal plasma was found to be slightly alkaline (7.4±0.1; 7.1-7.9; Table 1, 7). The concentration of spermatozoa was 417 ± 24.9 x 10^6/ml (Table 1; 7).

Table 1. Characteristics of Bactrian camel semen (after Mosaferi et. al., 2005)

	N	Mean±SEM	Min	Max
Time of Collection (min)	63	5.3±0.3	2.5	11
Volume (ml)	75	8.2±0.7	1.2	26
Osmolality (mOsm/kg H_2O)	53	318.2±1.9	300	348
PH	44	7.4±0.1	7.1	7.9
Concentration (X 10^6/ml)	52	417±24.9	133	945

Semen preservation

We were able to innovate an extender named SHOTOR diluent (SHOTOR means camel in persian language) for preservation of Bactrian camel semen (10). SHOTOR diluent consists of tris, 214.6 mM; citric acid, 64.2 mM; glucose, 66.6 mM, and fructose, 49.9 mM; with the osmolality of 330 mOsm/kg and pH of 6.9 (10).

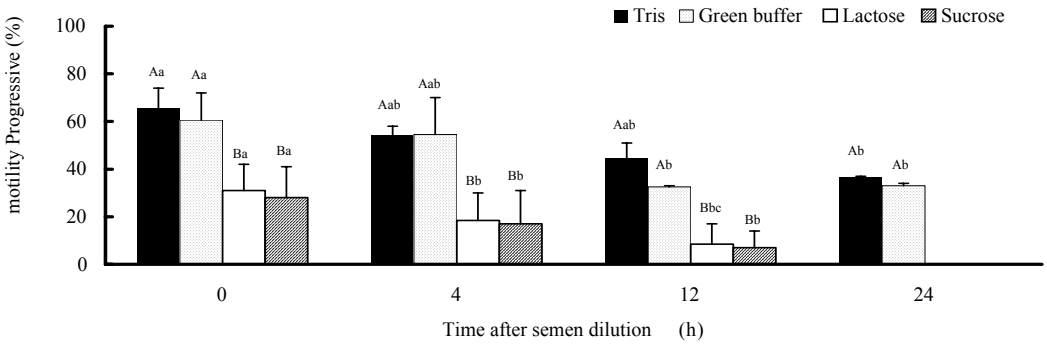

Figure 1. The effect of four semen extenders: SHOTOR diluent, Green buffer, lactose and sucrose on the progressive forward motility of Bactrian camel spermatozoa maintained at 4°C. ^{ab}Values with different superscripts indicate significant difference over the time within experimental groups ($P<0.05$). ^{AB}Values with different superscripts indicate significant difference at any particular time among experimental groups ($P<0.05$; Niasari-Naslaji et al., 2006)

Lactose and sucrose have been used for preservation of camel semen (3, 13). Our data indicated that lactose and sucrose are not suitable extenders for preserving Bactrian camel semen (10). Recently, we have compared 4 extenders including: lactose, sucrose, Green buffer and SHOTOR diluent for the short-term preservation of camel semen (10). As far as progressive motility of sperm concerns, after 4 hr incubation at 4 ºC, SHOTOR diluent was superior to lactose and sucrose extenders. It was also superior to Green buffer extender after 12 hrs incubation at 4 ºC (Figure 1).

Semen cryopreservation in the Bactrian camel is feasible when it is extended in SHOTOR diluent, cooled within 1 hr to 4 ºC, and glycerol was added, at the final concentration of 6% (9). Post-thaw progressive forward motility of spermatozoa was greater in SHOTOR (29.9%) diluent compared to IMV buffers (4.2%; $P<0.05$; Figure 2).

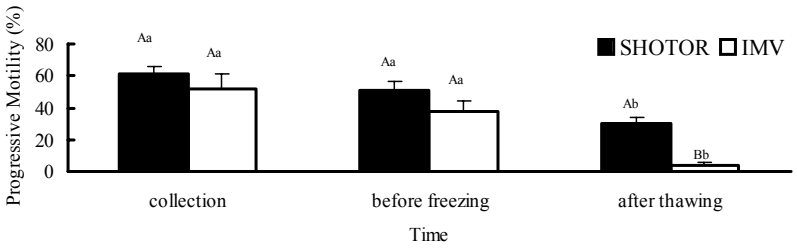

Figure 2. Comparing post-thaw progressive forward motility of Bactrian camel spermatozoa extended in SHOTOR diluent and IMV buffers. [ab]Values with different superscripts indicate difference over the time within experimental groups ($P < 0.05$). [AB]Values with different superscripts indicate difference ($P < 0.05$) at any particular time between groups (*Niasari-Naslaji* et al., 2006).

Follicle wave cycle

Camelids have follicle wave cycle rather than estrous cycle throughout breeding season. In Dromedary camel, the mature phase is 7.6 days, during which, the diameter of follicle reaches 13-17 mm in diameter (12). In Bactrian camel, the follicle mature phase is about 10 days during which the size of mature follicle reaches 13-19.7 mm in diameter (unpublished data). The inter-wave interval was about 19 days (unpublished) which could be reduced to 11.7±1.11 (9-14) days after induction of ovulation (11).

Control of ovulation

Ovulation can be induced by a single injection of GnRH analogues (Buserelin: 20 μgr, i.v.; 11; Alarelin: 25 μgr, i.m.; 6), hCG (1000-2000 i.u., i.m.; 2) or natural LH (Lutropin-v; 25 mg, i.v.; 6; LH; 300 i.u., i.m.; 2) in Bactrian camel.

Control of follicle wave cycle

Prostaglandin can not be used in camel due to the lack of functional CL during reproductive cycles. In addition, the beneficial application of progestogens is controversial in camel (4, 5, 11). However, two injections of GnRH, 14 days apart, made a tight synchrony of follicle wave emergence in Bactrian camel (11).

Interspecies embryo transfer

More recently we were able to transfer successfully Bactrian camel embryo to Dromedary camel. Accordingly, we achieved the first Bactrian camel calves born from Dromedary camels (unpublished data).

Conclusion

In conclusion, development of semen processing and preservation in association with controlling follicle wave cycle will assist us to extend AI network, as a valuable tool for breeding management and enhancing production of camel throughout the world. Interspecies embryo transfer provides a crucial approach to preserve endangered species of camelids from the threat of extinction and also to introduce new species of camelids to other country without introducing live animals.

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Developments in reproduction in dromedary camels

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Introduction

Reproductive efficiency of camels under natural pastoral conditions is low for reasons such as a short breeding season, long gestation period of 13 months and an 8 – 10 month period of lactation-related anoestrus. Therefore, a better understanding of reproductive physiology in the camel and the introduction of assisted reproductive techniques are important to try and increase the productivity of this species. This review looks at some of the developments in camel reproduction.

Reproductive physiology

All camelids are induced ovulators, usually ovulating only after mating, and if the camel does not conceive the corpus luteum has a very short lifespan of only 8 – 10 days. In the pregnant animal the embryo arrives in the uterus between days 5 and 6 and has to move rapidly around the uterus liberating its maternal recognition of pregnancy (MRP) signal before day 8 if it is to prevent luteolysis occurring. In ruminants, that have a cotyledonary placenta, this MRP signal is thought to be interferon tau (IFN-τ), whereas in the pig and horse, non-ruminants with a diffuse epithelialchorial placenta, fetal oestrogens secreted from the early conceptus are thought to be involved. The camel ruminates but has a diffuse epithelialchorial placenta, and studies involving culture of early embryonic tissue has shown that IFN-τ is not secreted, but large amounts of oestradiol-17β and oestrone are produced by the early embryo from day 10 after ovulation. This onset of oestrogen synthesizing ability coincides well with the observed time of luteolysis following a sterile mating, thereby suggesting that fetal oestrogens maybe an important part of the MRP signal (Skidmore et al, 2005). This hypothesis is further supported by the production of large multinucleate trophoblast cells that develop at frequent and irregular intervals along the trophoblast layer that, immunocytochemical staining studies have demonstrated, possess the enzymes necessary for the conversion of cholesterol to oestrogens.

The use of assisted reproductive technology in camels

As mentioned previously opportunities to improve the reproductive efficiency of camels are limited but artificial insemination (AI) and embryo transfer could be used to overcome these problems and provide the opportunity to produce more offspring from desirable sire and dam combinations.

Artificial insemination

As in other species AI is important in camels to enable more efficient use of genetically superior males, to help prevent the spread of venereal diseases and eliminate the need for transportation of animals. However there are only a limited number of studies involving AI in camels due to the difficulty in collecting, and the subsequent analysis and handling of semen. Semen is usually collected using an artificial vagina (AV) and typically an ejaculate is from 2 – 8ml in volume and has a very viscous consistency immediately after collection. This makes it difficult to mix with extender and thus sperm concentration and motility are difficult to assess. If the ejaculate is allowed to stand for 15 – 20 min it will partially liquefy and become easier to mix but the addition of trypsin or chymotrypsin had deleterious effects on the spermatozoa. For fresh and liquid storage of semen a number of extenders have been used but the best results to date in our laboratory have been achieved when the semen has been diluted (1:1) in Green Buffer (I.M.V. L'Aigle, France) plus 20% egg yolk (v:v 50 -60 % pregnancy rate).

Artificial insemination in camels requires the induction of ovulation as camels are induced ovulators. As ovulation occurs between 28 – 36h after GnRH injection the optimum time for insemination was thought to be 24 h after GnRH however, our studies have shown that insemination at the same time as GnRH injection has also yielded pregnancy results of 55%. Initial studies suggest that as many as $300x10^6$ live spermatozoa were needed for conception to occur but more recent studies have indicated that insemination of $150x\ 10^6$ live spermatozoa in the uterine body or just $80x10^6$ into the tip of the uterine horn ipsilateral to the ovary containing the dominant follicle, have both yielded pregnancy rates of 40–50 % (Skidmore and Billah, 2006). Diluted semen can be stored at 4°C for 24h and if the motility is at least 35 – 40% after 24h it can be inseminated. However, whilst pregnancy rates of 55% are obtained with fresh semen, they are reduced to around 25% with cooled semen (Bravo et al, 2000).

Embryo Transfer

The technique of embryo transfer is also important to produce more genetically desirable offspring. In the dromedary camel methods for superovulating donor animals and for collecting and transferring fresh day 7 blastocysts into synchronized day 6 recipients are well established. However because camels are induced ovulators, synchronization of groups of recipient animals can create problems owing to the absence of a cyclical CL. Synchronization of recipients can be achieved by selection of recipients from a random group of cyclic camels which involves examining their ovaries regularly and injecting all those with a mature follicle in their ovaries with GnRH 24h after the donor has been mated. Alternatively recipients can be treated with progesterone–in-oil for 10 days and then injected with 1500i.u. of eCG. Progesterone treatment should stop the day of eCG treatment in the donor animals and the eCG then guarantees the presence of mature follicles in the ovaries at the same time as the donor. More recently giving two GnRH injections 14 days apart has also been shown to synchronize camels as follicles tend to ovulate, luteinize or regress after the first GnRH and then another mature follicle is present 14 days later. The second GnRH needs to be timed to be given to the recipients the day after the donor is mated.

It is not always possible however, to synchronize recipients with donors so recent studies have examined the effects of progesterone or meclofenamic-acid treatment in asynchronous recipients. Daily intramuscular injections of progesterone (100mg/day) can be given to non-ovulated recipients or to recipients that have ovulated ahead of the donor, starting 2 days before embryo transfer. However, as there is no CL in non-ovulated animals and the CL is likely to undergo luteolysis before the embryo is established in those recipients ovulating ahead of the donor, this means daily progesterone injections would be needed throughout pregnancy. However, if daily progesterone injections are started in recipients that are only 2 days post ovulation and the embryo is transferred on day 4, then progesterone treatment can be stopped on day 12 after ovulation as the embryo has established itself and secreted enough maternal recognition of pregnancy to maintain the CL itself. Luteal lifespan in camels can also be prolonged using meclofenamic-acid, a prostaglandin synthetase inhibitor. If meclofenamic-acid is administered orally from day 7 after ovulation until 7 days after embryo transfer and embryos transferred on days 8, 10 or 12 after ovulation then pregnancy rates of 80%, 60% and 70% have been achieved respectively, as compared to 10% in untreated day 8 control recipients.

Cryopreservation of camel embryos

The ability to be able to cryopreserve camelid embryos is important to enable the storage of good camel genetics and to eliminate the need to synchronize recipient animals with the donor, as embryos can be stored and transferred after naturally timed ovarian cycles. Day 6 and 7 camel embryos have been cryopreserved by vitrification and slow cooling methods with limited success.

Pregnancy rates of 35 – 40% have been obtained using slow-cooling protocols were day 7 embryos were exposed to 10% ethanediol for 10 min before being loaded into a embryo freezing machine (IMV Technologies) at -7°C and cooled at -0.5°C/min to -33°C. After storage they were thawed and rehydrated by expelling into holding media containing 0.2M sucrose for 5 mins. For vitrification, day 6 and 7 embryos were exposed to vitrification solutions (20% glycerol+20% ethanediol+0.3M sucrose+0.375M glucose and+3% polyethylene glycol) in three steps and after loading into 0.25ml straws were plunged into liquid nitrogen. Embryos were thawed and rehydrated in 0.5M sucrose and 0.25M sucrose in HM (5 min each step) but a greater number of the day 7 embryos (56%) were fractured or torn after thawing and rehydration, and no pregnancies resulted from the 9 embryos transferred. Better survival rates were obtained with the day 6 embryos (94%) which resulted in a total of 8 fetuses from 21 embryos transferred.

Micromanipulation of camel embryos to produce identical twins

The production of identical twins in science is particularly valuable because the results of the treatment of whatever one twin receives and not the other, is directly related to that treatment. Identical twin camels have been produced by embryo bisection of day 6 embryos using an AB splitting blade fitted to the motorized arm of an Eppendorf micromanipulator. Each half is transferred to a day 6 recipient and to date two sets of identical twins have been born in the UAE.

These results demonstrate that with good management, controlled breeding and use of assisted breeding techniques it should be possible to increase the reproductive efficiency of these animals.

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Advances in reproductive biology in South American Camelids

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With the aim of optimizing reproductive management of genetically superior animals, application of assisted reproductive techniques (artificial insemination, embryo transfer, in vitro fertilization, etc.) offers the possibility of increasing genetic progress and reproductive efficiency in these species.

Male

Semen collection and ejaculate processing

Obtaining, processing and conserving semen plays a fundamental role in applying reproductive biotechnologies. With the intention of using assisted reproductive techniques to obtain embryos from ejaculates rather than from epididymis, our laboratory reached the following objectives: 1) we designed a semen collection method using electro ejaculation (EE) that allowed us to repeatedly obtain ejaculates of equal or superior quality to those obtained using an artificial vagina (AV) from all males used (Director et al., 2007); 2) a protocol that permitted improvement of seminal characteristics in llama was designed (Giuliano et al., 2007) and 3) the rheological characteristics of seminal plasma were studied in llamas (Miragaya et al., 2008).

Briefly, the EE protocol consisted of the following steps: 1) measurement of the distance from anus to prostate in each male; 2) inducing urination immediately before semen collection; 3) sedation and general anaesthesia and 4) electrical stimulation. Ejaculates were collected from 100% of the males (6/6) using EE and 85% of the males (5/6) using AV. Ejaculates obtained with EE showed a greater volume and greater percentages of spermatozoa that were motile, live and showing swelling (Giuliano et al., 2008). To date in our laboratory we have collected a total of 230 ejaculates from 12 different males using EE, with an efficiency of 98%.

Sample improvement

Regarding improvement of ejaculate characteristics, the results of a descriptive study carried out on ejaculates incubated in H-TALP-BSA with 0.1% colagenase

showed that with this treatment, ejaculate viscosity was decreased and progressive motility was induced while maintaining fertilizing capacity (Giuliano et al., 2007). Regarding the rheological characteristics of 22 ejaculates obtained from 10 llamas, the apparent viscosity coefficient (AVC) was measured at 37° C using a cone plate CP 42 rotational viscometer (Miragaya et al., 2008).

Female

Inhibition of follicular activity and ovarian superstimulation

It has been shown that initiation of a treatment in the presence of a follicle greater than 5 mm induces the growth of only that follicle (Miragaya et al., 2006). An intravaginal device impregnated with 0.33 g of progesterone (CIDR®; Eazi-Breed® Pharmacia & Upjohn, New Zealand) was used in each female over a period of five days (Chaves et al. 2002). On the day of CIDR® insertion, all females received a single dose of 1 mg of estradiol benzoate (Trasorras et al., 2005). We observed that after inducing an artificial luteal phase with EB and CIDR®, a dose of 500 IU of eCG (Novormon®, Syntex, Argentina) did not produce ovarian superstimulation but both 1000 and 1500 IU of eCG were effective; applying 1500 IU follicle production is greater and so is the recovery of cumulus-oocyte complexes (COC's) (Table 1) (Trasorras et al., 2007a). Using buserelin at the end of the superstimulatory treatment with eCG was beneficial for recovering a larger quantity of expanded COCs, which can then be used directly in assisted reproductive techniques such as IVF and ICSI, without needing previous maturation (Trasorras et al., 2007a).

Table 1: Total ovarian response after eCG and buserelin treatment. Values with different superscripts within the same row differ significantly ($p<0.05$).

	eCG treatment group	
	1000 IU (n = 10)	**1500 IU (n = 9)**
Nº aspirated follicles (mean ± SD)	83 (8.3 ± 3.7)[a]	137 (15.2 ± 7.5)[b]
Nº COCs recovered (mean ± SD)	69 (6.9 ± 3.3)[a]	112 (12.4 ± 6.1)[b]
Percentage of recovery	83.1 %	81.7 %
Degree of maturation:		
– Compact	7/69	14/112
– Expanded	56/69	77/112
– Denuded	6/69	21/112

Obtaining embryo in vivo

To maximize ovarian response in the female donor, an ovarian superstimulation treatment was applied using 1000 IU of eCG when follicles of ≤ 5 mm were present. Mating with a fertile male was carried out when two or more dominant follicles (≥7 mm) were observed and this was repeated 24 h later with a different male, to reduce the male effect. Transcervical uterine flushing was carried out on day 8 after the first mating. Response to superstimulation was 90% (9/10) and the rate of embryo recovery was 66.7% (6/9). The average number of embryos recovered per female was 2.4 ± 0.8 (mean ± SEM) (Carretero et al, unpublished).

In vitro embryo production

In vitro embryo production demands a large quantity of oocytes capable of being fertilized. COC's can be obtained from more than 80 % of the follicles aspirated by laparotomy in llamas (Trasorras et al., 2007a). There are few publications on IVF and ICSI in camelids. As previously mentioned, the first IVF was carried out by Del Campo et al. (1994) using epididymal spermatozoa. Conde et al. (2007) obtained a larger production of blastocysts by IVF using raw semen and no capacitating agents (see Table 2). In 2003, our group reported the first embryos produced by ICSI in llama but in this study, injected oocytes were not subjected to artificial activation (Miragaya et al., 2003). Incubating injected oocytes in ionomicyn and DMAP induced artificial activation. According to the latest research, the greatest blastocyst rate was obtained using ICSI without artificial activation (Conde et al., 2007). This study represents the preliminary development of these two techniques using raw llama semen processed with 0.1% colagenase and, for the first time, *in vitro* produced expanded blastocyst embryos were obtained (Table 2).

Embryo transfer (ET)

The procedure of manual stabilisation of the cervix when passing the AI pipette and transcervically depositing PBS in the uterine horns or uterine body do not produce a decrease in plasma levels of progesterone (Trasorras et al., 2007b). ET was carried out in recipient females 6 days after ovulation, because up until that day $PGF_{2\alpha}$ levels are still basal (Aba et al., 2000). Inovulation of the left uterine horn with a corpus luteum in the ipsilateral ovary showed the highest pregnancy rate (50 %) (Trasorras et al., unpublished).

Table 2: *Lama glama* embryos produced by IVF with or without capacitating agents, and by ICSI with or without artificial activation.

Groups	Cleaved (%)	Blastocysts (%)	
		Blastocysts/cleaved	Blastocysts/oocytes
IVF	20/49 (40.8)		
With capacitating agents		7/20 (35)	7/49 (14.3)
Without capacitating agent	19/45 (42.2)	9/19 (47.4)	9/45 (20)
ICSI			
Activation with ionomicyn and DMAP	9/25 (36)	4/9 (44.4)	4/25 (16)
Without activation	2/27 (7.4)	1/2 (50)	1/27 (3.7)

The development and application of assisted reproductive techniques in camelids allows us to increase our knowledge on the fundamental mechanisms that intervene in fertilization and to increase reproductive efficiency, both in the domestic and wild camelid species.

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The development of collection, processing and storage technologies for alpaca semen

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Introduction

Artificial insemination (AI) is widely used in animal breeding programs. At present the technology is poorly developed for Camelids owing to the difficulty in collecting high quality semen and its viscous nature. There have been several reviews of the reproductive physiology of male Camelids (Brown 2000; Sumar 1996; Tibary and Vaughan, 2006). The fundamental differences between other domestic animals and Camelids are the latter's lower sperm production, extended mating length and unusual ejaculate characteristics.

The problems of lengthy mating in Camelids have been largely overcome. There have been several studies on the collection of semen from alpacas (reviewed by Vaughan et al., 2003), the majority concluding that an artificial vagina (AV) fitted inside a wooden mannequin covered in a tanned alpaca hide is the most suitable method. The AV and collecting container are kept warm by wrapping in a small electric blanket. Despite these developments, ejaculates collected by AV are often of low volume/sperm concentration, limiting the number of AI doses that can be divided from each ejaculate.

The main barrier to the development of AI and semen preservation technology in Camelids is the viscous nature of the ejaculate. This makes semen samples hard to divide into aliquots, treat with common stains and smear on slides (to assess sperm quality) and dilute with extenders (heterogeneous mixing results in poor post-storage motility of sperm). The viscous seminal plasma has been reported to result from the presence of mucopolysaccharides (Garnica et al. 1993; now referred to as proteoglycans or glycosaminoglycans). There is no published evidence to support this claim.

Given these constraints, this paper summarises our studies on the collection, processing and storage of alpaca semen for AI and our attempts to ameliorate the problem of the viscous seminal plasma so the semen may be stored in a liquid or frozen state.

Sperm production

Sperm production is a function of testis size in most domestic species. Llama and alpaca testes are small, accounting for 0.01 and 0.02 – 0.5 % of body weight, respectively, compared with 1.25% for rams (Setchell, 1978). Testis size and therefore sperm production rates can be increased to some extent through nutrition. However, no relationship has yet been established between testis size and sperm production in alpacas (Bailey et al., 2007).

Collection methods

In alpacas, there is considerable variation between males and between ejaculates within males in semen volume and sperm concentration (Vaughan et al., 2003; Morton et al., 2008).

We have investigated the effects on alpaca semen viscosity and quality parameters of (1) adding a cervix-like structure to the silicone liner of the AV, (2) the presence of females, (3) collecting semen into Androhep® (Minitüb, Germany), skim-milk or Tris-based diluents, and (3) supplementing the Tris collection diluent with catalase (0, 100, 200 or 600 units/mL). The cervix-like structure in the AV increased mating length, whilst the presence of females during semen collection did not improve semen quality. Collection of semen into Tris diluent improved sperm motility (58.0 ± 11.9 %) compared with the control (34.0 ± 10.8 %; $P<0.05$), Androhep® (33.5 ± 10.7 %) and skim-milk diluents (28.2 ± 10.4 %), whereas collection into skim-milk improved acrosome integrity compared with controls (93.2 ± 1.3 vs. 83.6 ±3.1 % intact). Semen viscosity was reduced by collection into Androhep® (4.6 ± 1.7 mm) and skim-milk diluents (3.6 ± 1.3 mm) compared with Tris diluent (5.7 ± 2.1 mm) and no collection medium (9.3 ± 3.5 mm; $P<0.05$). Supplementation of Tris diluent with catalase increased semen viscosity (4.2 ± 2.7, 5.0 ± 3.2 and 4.9 ±3.2 mm for 100, 200 and 600 units/mL, respectively) compared with unsupplemented controls (2.2 ± 1.4 mm). Collection of alpaca semen by AV into Tris diluent increased semen quality suggesting benefits might be obtained from further work on collection media for alpaca semen.

Dealing with the viscous seminal plasma and storage of liquefied diluted semen

A number of mechanical treatments (centrifugation, density gradient centrifugation, needling and pipetting) and enzymes (0.5, 1.0, 2.0 and 4.0 mg/ml of Papain, Collagenase and Trypsin in the diluent) were studied for liquefaction of the

viscous semen. All enzymes reduced the viscosity of semen but satisfactory motility was only maintained (for 1 hr) after addition of 0.5 mg/ml Papain or Trypsin (viscosity: 1.3 ± 1.0 vs. 10.8 ± 3.0mm, $p<0.05$; motility: 47.5 ± 7.1 vs. 40.5 ± 6.2%; normal morphology: 64.6 ± 5.5 vs. 56.6 ± 3.4% for Papain vs. controls, respectively), whereas Collagenase was toxic at all concentrations tested (< 4% motile sperm within 10 minutes of addition of enzyme vs. 49.0 ± 3.6% for controls). All enzymes, whether added during or after semen collection, were detrimental to the acrosome integrity of sperm (41.6 ± 14.3 vs. 95.0 ± 2.6% intact acrosomes for Papain vs. controls, respectively). Motility of sperm was maintained after liquefaction by the mechanical methods of centrifugation and density gradient centrifugation but the proportion of sperm recovered was only 60-70% compared with other methods. Needling did not reduce semen viscosity. However, pipette mixing of semen during dilution with handling medium reduced semen viscosity (2.6 ± 0.3 vs. 11.7 ± 1.8 mm), with improved motility (62.8 ± 1.4 vs 48.6 ± 3.2%, $p<0.05$) and without detrimental effects on acrosome integrity of sperm (90.4 ± 0.9 vs 92.4 ± 0.9%, for after vs. before pipetting, respectively).

For liquid-stored semen, the highest motility of sperm was obtained when semen was liquefied during dilution (1:4) with Biladyl® (Minitüb, Germany) and stored at 4 °C. Sperm remained viable for periods up to 96 h although optimal storage times remained within 48 h of collection. Epididymal sperm diluted in Lactose diluent and pellet-frozen on dry ice had superior post-thaw survival compared with the other cryopreservation methods examined (Morton et al., 2007). To date we have been unable to obtain pregnancies after transcervical AI of alpaca females with frozen-thawed epididymal sperm at an induced ovulation.

Conclusions

Efficient methods have been developed for collecting high quality alpaca semen using an AV. Addition of Androhep® and Skim-milk to the collecting glass reduces semen viscosity but Tris diluent increases sperm motility without influencing viscosity or other semen quality parameters. The gelatinous component of the alpaca ejaculate can be removed by pipetting with the processing medium while maintaining sperm motility and integrity. The function of ejaculated sperm can be maintained up to 96 h of chilled storage and that of epididymal sperm after cryopreservation in appropriate media. While the development of artificial reproductive technologies is underway in

alpacas, and there has been success in the preliminary development of a number of procedures, there is still considerable future research required before these technologies can be commercially applied. Nevertheless, collection of higher quality, lower viscosity semen using the procedures outlined in our studies could be a significant advancement facilitating the development of AI technology in alpacas.

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Experiences in artificial insemination of llamas and alpacas

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Introduction

Advances in artificial insemination of llamas and alpacas have been slow mostly due to an inadequate semen collection method (Bravo et al., 1999). Reports on artificial insemination have appeared since 1968, but lately at least three Peruvian institutions have been working on this issue and efforts in other countries as well. The advantage of artificial insemination over other reproductive technologies is that an outstanding male can be used for many more females than in natural breeding. The tremendous growth of the dairy industry is due to the application of artificial insemination many years ago. The genetic improvement in milk production of cows could be copied for llamas and alpacas. Instead of milk, though, it would be to perpetuate solid fleece colors besides white, and also outstanding fiber characteristics. This paper is a short summary of the research conducted on artificial insemination of llamas and alpacas at La Raya Research Center at Cusco University in Peru. Aspects of semen collection using a dummy, semen dilution, semen preservation, and use of frozen-thawed semen in females are reviewed briefly.

Materials and Methods

Semen collection: There are many methods for collecting semen, but so far the best method is using an artificial vagina accommodated inside a dummy in sternal position. The artificial vagina has to be kept warm for 20 to 25 minutes (comparable to natural breeding) and there should be a simulation of the cervix of the female. Keeping the artificial vagina within an electric blanket is one of the answers. In places where there is no electricity, a pre-warmed gel could be used to cover the artificial vagina. The cervix is simulated by making a stricture in the linen. A coiled 2-3cm electrical cord outside the latex liner is sufficient to simulate a cervix. When the male senses that his penis is threading the natural cervix, he starts to ejaculate.

This method mimics the natural use of a female, so the only thing to overcome is the male's resistance. For this purpose the male is trained to breed the dummy with the

artificial vagina fitted inside and thus the male can be used to collect semen. There is no change in semen characteristics using this method. The semen is ready for evaluation and dilution.

Results and Discussion

The main semen characteristics of alpaca collected with an artificial vagina are as follows: ejaculated volume 0.9 – 2.3 mL, motility, 14 to 80%; spermatic concentration 56 to 614 million per mL; live spermatozoa 54 to 85%; normal spermatozoa 54 to 82%. In general, semen characteristics are similar to those of other livestock species with the exception of motility and concentration. Motility is not progressive like in bulls and rams; rather, individual spermatozoa move slowly due to the presence of a thick, almost gel-like seminal plasma. Spermatozoa are entrapped in seminal plasma, and individual spermatozoa can be observed moving slowly and resembling tadpoles. Llama and alpaca semen is extremely gelatinous, especially in the first ejaculation. Spermatic concentration is lower than that of bulls and rams. In those species, there are billions of spermatozoa per mL, but in llamas and alpacas, spermatic concentration runs in the millions per mL.

Semen dilution

Before dilution, semen should be a liquid, not a gel. The use of hydrolytic enzymes has facilitated the elimination of the gel structure. The first attempt to degelify semen was done using trypsin. Trypsin (1:250) was effective; trypsin 1:500 was partially effective; and greater dilutions (1:1000 and 1:2000) were not effective at all. Trypsin in powder, diluted from a stock solution of 2.5% to 1.25, 0.6, and 0.3%, were also efficient. A second enzyme used was collagenase. Warmed collagenase (1mg/mL) has given the best results. It should be added slowly through the walls of the collecting tube and mixed with semen. Although collagenase at 10 and 20mg/mL eliminated the gel matrix, it affected significantly the acrosome integrity of spermatozoa. Collagenase is very effective. It's rapid, and its action is irreversible (Bravo, Ccallo, Garnica, 2000). It takes only 2-3 seconds for semen to become liquid and thus ready to be diluted.

Semen preservation

The best semen extender so far is Tris buffer (Baca, 1998). It works far better than skim milk, glucose citrate, egg-yolk citrate and other extenders. The first study on

extending semen and maintaining it at 37 degrees C was done by testing phosphate buffer, skim milk, and egg-yolk citrate. The percentages of live spermatozoa at collection times were 61.1, 60.8 and 64%, respectively. After 2 hours those percentages were 22.2, 15.6, and 35.4, respectively. As far as length of time, for semen samples maintained at 4 degrees C over a 72-hour period, egg-yolk citrate maintained 70% live spermatozoa by 48 hours and skim milk maintained 68%. Glucose-citrate had 60% live spermatozoa by 24 hours. For freezing purposes, Tris buffer (70%) was better than triladyl (65%), and egg-yolk glucose citrate (58%) in maintaining live spermatozoa.

The effect of glycerol as a cryoprotectant agent was also determined at the time of thawing the semen (Salinas, 1999). Different glycerol concentrations -- 0, 15, 3.75 and 7.5% -- were added to alpaca and llama extended semen samples with Tris buffer. The best glycerol concentration was 7.5%, which resulted in 32.6% of live spermatozoa. The percentage of live spermatozoa was 20% for glycerol concentrations of 0, 1.5, and 3.75%. Thus, 7.5% glycerol, as with other livestock species, has given adequate results in the process of freezing.

Llama and alpaca semen, loaded into straws, has been frozen using vapors of liquid nitrogen. Slow freezing appears to work better than plunging the straws into liquid nitrogen. When straws were used, it could be clearly observed that they change in color, from yellow at the initiation of freezing to pale yellow when the semen is fully frozen. At that point, semen straws are ready to be loaded into goblets, accommodated into the canister, and maintained in liquid nitrogen.

Artificial insemination

Artificial insemination of llamas and alpacas has been done using undiluted semen, extended semen, refrigerated semen (Castillo, 1997), and frozen-thawed semen (Table 1). The minimum number of spermatozoa per straw has been also determined. An adequate fertility rate (60%) has been reported using 8 to 12 million spermatozoa per straw. By contrast, 53% fertility was reached when 4 million spermatozoa were used per straw. In general, 35% of inseminated llamas became pregnant, in comparison to 60% of alpacas when frozen-thawed semen was used. These results are promising and they will be improved with time. Artificial insemination per se could be done by two methods: cervical and uterine.

1) The cervical method, as in cows, involves holding the cervix via rectum with one hand, and threading the insemination pipette into the uterine horns with the other hand.

This technique should take into account the hand size of the operator. A hand that fits a size 7.5 surgical glove is the maximum size that will work in the rectum of alpacas. Fertility rates varied between 45 and 65% using this method.

2) The uterine method uses laparoscopy and semen deposition into the uterine horns through a blunt perforation of the uterine wall. The needle is attached to an insemination pipette and semen is deposited into the uterine horn ipsilateral to the ovary containing the ovulatory-sized follicle. This method requires the use of a portable surgical table, a laparoscope, some surgical equipment, and some medicines to sedate the alpaca.

Table 1. Different protocols of artificial insemination in llamas and alpacas

Semen	Extender	Ovulation induction	Females inseminated	Fertility (%)	Authors
Fresh	Non extended	hCG	40	67	Bravo and Flores, 1994
Fresh	Egg-yolk glucose citrate	hCG	80	57	Bravo and Quispe, 1995
Fresh	Saline phosphate	Vasectomized	133	40	De la Vega, 1996
Thawed	Egg-yolk glucose citrate	hCG	19	26	Bravo at al. 1996
Fresh	Tris buffer	GnRH	25	69.6	Bravo at al. 1997
Refrigerated	Tris buffer	GnRH	25	66.7	
Thawed	Tris buffer	GnRH	25	68	

To be done properly, artificial insemination has to consider the reproductive physiology of the female. An ovulatory-sized follicle (7 mm) should be present, and ovulation should be triggered either with a vasectomized male or the application of luteinizing hormone. The hormones that may be used are: hCG and GnRH. The dosages are: 750 IU for hCG and 80-100 μg of GnRH per female. Ultrasonography is used to detect the size of the ovarian follicle. Rectal palpation is a second alternative by which an experienced operator can determine the size and location of the ovarian follicle.

Conclusion

In summary, significant advances have been made in the artificial insemination of llamas and alpacas. Semen collection using an electric blanket and a simulated cervix is feasible and reliable. Semen dilution works better with Tris buffer. Semen freezing is a

reality that took many years to develop. Artificial insemination can be done using fresh, refrigerated or frozen-thawed semen. The minimum number of spermatozoa for insemination is 8 to 12 million per dose. If possible, semen should be deposited ipsilateral to the ovary with the ovulatory-sized follicle. Artificial insemination with frozen-thawed semen opens new developmental horizons for llamas and alpacas.

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Why artificial insemination in dromedaries is not successful?

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Introduction: Demands of a particular racing male camel for breeding females could reach up to 1500 females per year, impossible to fulfill with natural service (Tibary and Anouassi, 1997), hence there is need of Artificial Insemination.

Artificial collection of semen: Artificial collection of semen can be successfully accomplished during breeding season when males are in active phase of rut. It was not convenient to accomplish it during non-breeding season. Sexual libido was high during January to April, declined slowly Over May followed by complete cessation during later half of June. Sexual libido was almost negligible during July to November. Sexual libido was also low during December. Month wise Copulation time and volume of semen ejaculated by 6 male camels for 6 months of breeding season have been shown in Table 1. Mean copulation time within animals did not differ significantly over different months, while it differed significantly between animals. Volume of semen ejaculate differs significantly between animals but within animals it was significantly different over different months in only 1 animal while remaining 5 animals did not exhibit month wise differences. Overall average copulation time and volume of semen ejaculated were measured as 295.73 ± 11.82 seconds and 2.85 ± 0.21 ml, respectively. Absolutely no difficulties are confronted in artificial collection of semen if the males are well trained for this purpose.

Characteristics of semen: A characteristic semen ejaculate is thick, gel like, frothy and off-white to sparkling white in colour. Thick, gel like ejaculate can be considered as satisfactory, while thin ejaculates even though might measure more in volume may indicate an incomplete ejaculate. An intriguing aspect is that semen ejaculate does not mix with the semen extenders.

Liquefaction of semen: A thick characteristic semen ejaculate liquefy only if it is kept to room temperature, while it does not liquefy if it is extended, cooled and preserved at refrigeratory temperature immediately after collection.

Mass activity evaluation: Mass activity evaluation is not feasible in camel apparently due to thick consistency and motionless state of spermatozoa.

Table 1: Month wise mean copulation time and volume of semen ejaculated.

Month	Copulation time (Seconds)	Volume of semen ejaculate (ml)
December	216.8 ± 22.74	1.7 ± 0.29
January	272.51 ± 20.9	2.13 ± 0.29
February	300.13 ± 22.86	2.5 ± 0.28
March	395.95 ± 42.42	4.76 ± 0.98
April	320.32 ± 30.71	3.78 ± 0.66
May	245.85 ± 31.41	2.76 ± 0.30
Overall	295.73 ± 11.82	2.85 ± 0.21

Effect of rubber funnel contact on camel spermatozoa: There has been a common belief among the scientists that contact of rubber funnel has a lethal effect on the spermatozoa and they gets paralyzed as a result become motionless or immotile. But our observations have revealed no lethal effect of rubber funnel contact.

Individual motility evaluation: Individual motility examinations performed on semen diluted in physiological buffers have revealed that variable picture of oscillating spermatozoa, progressively motile spermatozoa and even motionless spermatozoa in different fields of same slide could be seen. Structures apparently like sperm depot can be visualized in diluted semen. Spermatozoa evacuating from these sperm depot have been visualized. Sperm depot is maintained for prolonged periods.

Sperm concentration: Since spermatozoa are stored in unique sperm depots and semen ejaculate is not miscible in buffers, a uniform distribution of spermatozoa can not be ensured, as such the validity of sperm counts of camel semen appears doubtful.

Refrigeratory preservation of camel semen: Using Tris and egg-yolk dilutor, semen could be preserved at refrigeratory temperature and sperm motility is maintained in cooled semen for 48 hrs in majority of the samples, 72 hrs and even for few weeks in some samples.

Cryopreservation of camel semen: Pre freeze motility varied from 47 to 70% in different males. Post thaw motility declined from 23.5 to 47.5% in individual semen samples with an overall estimated loss of 62.5% of the progressively motile spermatozoa due to freeze thaw process. Based on criteria to approve semen of 30% or greater post thaw motility, only 37% of semen samples processed in present study

qualified for approval to be of use for AI. The rejection rate of more than 50% was greater than those of dairy bulls of 5-15%. Post thaw motility of same semen sample cryopreserved in duplicate vials in same batch differs significantly. Post thaw duration of survival of thawed spermatozoa studied at 37 and 4°C revealed that the percent decline was 17, 30, 35.8, 44.1 and 65.5%, at 1, 2, 3, 4 and 24 hr of incubation at 4°C, while at 37°C, the reduction in motility was about 50% after 1 hr. At 2, 3, 4 and 24 hr after incubation, almost 93, 99 and 100 % spermatozoa lost motility.

Artificial insemination of female camels: Large numbers of artificial inseminations were attempted using frozen thawed, diluted cooled and neat freshly collected semen. A total of 8 female camels could be artificially impregnated over several years. The success rate has been too low. All possible modifications were attempted to maximize the pregnancy rate but without much success.

Initially female camels in follicular phase were inseminated after exogenous administration of hCG for induction of ovulation but without much success. It was observed that some of the seminal fluid deposited into the uterus of females flow back out through the vulva. Later, neat semen was deposited into the uterus so that it sticks to the genitalia and does not flow back. Three females could be impregnated over a large number of attempts. Next year another scientist repeated the AI experiment with no success. In succeeding year a modification like mechanical stimulation of cervix was also introduced in addition to hCG administration, but only one female could be impregnated. Progesterone profiles were also monitored twice weekly after insemination and results showed that 4/10 females have ovulated. In this way it was speculated that ovulation rate is also low but impregnation rate is much lower than ovulation rate. In year 2006-2007, two trials on artificial insemination of female camels with or without sedation and hCG resulted in 3 impregnation out of 13 attempts in first group with sedation and hCG and no pregnancy out of 4 attempts in group 2 without sedation and hCG. All the 3 artificially impregnated females have calved this year. This year 7 female camels that failed to settle during natural breeding program of the herd were artificially inseminated with freshly collected neat semen after serving with a vasectomized teaser. None of these females conceived. These results showed that pregnancy rate with artificially inseminated female camels are too low to be of any practical utility. Vaughan (2003) reported results comparable to those of present study, no pregnancy out of insemination of 24 female alpaca. But Anouassi et al, 1992, Bravo

et al, 2000; Skidmore and Billah, 2006 and Medan et al, 2008 have reported success in artificial impregnation of female camels. It is very difficult to point out as to what may be the probable reason for failure of artificial impregnation but low ovulation rate can be an important cause. Recent experience of breeding management of camel herd has revealed that unexplained sort of sub fertility and sterility prevails in a significant number of males. The pregnancy rate of 0/20, 0/19, 2/9, 1/6 and 1/10 services for 5 male camels under natural service indicated low fertility rate in this species.

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Characterization and conservation of Maghrabi camel semen

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Introduction

Semen preservation is an essential tool to extend artificial insemination programs and to maintain the genetic potential of the male. The progress in semen preservation and related techniques in the camelidea family still slow in comparison to other livestock species. This, in turn, prevents extensive use of artificial insemination in this family. This could partly be due to the lack of proper semen extender to maintain the viability of spermatozoa for short and long term. In this study, we tested 5 diluents for short term conservation and/or cryopreservation of Maghrabi camel semen.

Materials and methods

1. Camels

Three Maghrabi camels (*Camelus dromedarius*), aged 9–10 years and weighting 480–604 kg were used for semen collection twice a week (Monday, Thursday) during breeding season (January-March). During the study, ambient temperature varied between 10 and 25°C. On a daily basis, each camel received 5 kg oat hay and 5 kg mixed concentrate (including 45% barley, 30% olive cake and 25% wheat bran). Wheat straw was used as litter. Water was offered ad libitum.

2. Experimental design

Preparation of extenders: Five extenders (D0, D1, D2, D3 and D4) were tested in this study. Four (D1-D4) were elaborated in the laboratory (table 1) and one (D0: Bioxcell® I.M.V) was a commercial extender for bovine conservation semen. Extenders 1 and 2 were used for freezing and diluents 3 and 4 were used for cooling. Extender 0 which was prepared as manufactory instructions was used for freezing and cooling.

Semen collection, processing and evaluation: Semen was collected by a modified bovine artificial vagina. The anterior end was connected to a graduated, 15 ml glass tube. The entire AV, including the glass tube, was wrapped with a heating pad to maintain warmth (40-45°C) during the semen collection period and to protect the semen from light. Duration of copulation was recorded (from the time of penis intromission into the AV until withdrawal). Following semen collection, ejaculate volume, consistency, color, smell and pH were recorded directly and glass tube was protected as soon as possible by aluminum paper, filled and placed in room temperature (20°C). In order to reduce the viscosity for further evaluation, semen was manually stirred 4 to 5 times during 40 min. After that, sperm concentration was determined and expressed as number of spermatozoa per ml. The total number of live and dead spermatozoa was assessed using the eosin B-fast green vital staining technique. A total number of 200 sperms were counted with color screen related to a light microscope (400×) and the percentage of live spermatozoa was determined.

Table 1: Composition of elaborated extenders

	Long term conservation		Short term conservation	
Composition	Extender 1	Extender 2	Extender 3	Extender 4
Half skimmed milk UHT (ml)	-	-	100	-
Glucose (g)	5.67	0.75	-	3
Lactose (g)	-	-	-	0.5
Raffinose (g)	-	-	-	0.25
Fructose (g)	-	-	-	0.10
Glycine (g)	-	0.93	-	0.10
Distilled water (ml)	77.5	77.5	-	100
Egg yolk (ml)	22.5	22.5	-	10
Skimmed milk (ml)	-	-	-	100
Penicillin (UI)	20	20	10	10
Streptomycin (UI)	20	20	10	10
Gentamycin (mg)	10	10	5	5
Citric acid (g)	-	1.35	-	-
Glycerol (ml)	4.0	4.0	-	-
Tris (g)	-	2.43	-	-

Short term conservation and cooling procedure: After evaluation, ejaculate was sampled in three equal fractions and then extended in the short term conservation and cooling diluents (D0, D3, D4) at a final concentration of 150 x 10^6 SPZ/mL. Diluted semen was then sampled in 0.25 ml straws (IMV). One half of obtained straws was kept in dark room at ambient temperature and the rest was putted in cardboard box in 4°C.

The viability parameter of sperm was evaluated for each treatment after 3, 6, 24 and 48 h (3 straws/treatment).

Freezing procedure: Semen was sampled in three equal fractions and extended in the freezing diluents (D0, D1, D2) at a final concentration of 150 x 106 SPZ/mL. Diluted semen was then sampled in 0.25 ml small straws and cooled during 1 h to 4 °C. Then, specimens were placed on a rack standing at 5 cm above the surface of liquid nitrogen during 5 min and at the surface of liquid nitrogen during 5 min, after that straws were immersed into liquid nitrogen. After 1 week, four thawing procedure (6" at 55°C, 8" at 55°C, 12" at 55°C et 40" at 40°C) were tested.

Results and discussion

Results are presented as percent mean ± S.E. Data were arcsin transformed before analysis. The Maghrebi camel has a white greyish color sperm with an acrid odor and a basic pH (8.01 ± 0.25). The copulation in this species lasts 5.7 ± 3.1 min, this value is compared to that (4.5 min) reported by Aminu et al. (2003). The ejaculate volume varies from 1 to 15 ml with an average of 4.2 ± 3.0 ml. Tibary and Anouassi (1997) had observed a value varying between 2 and 12.6 ml. The concentration of the spermatozoa was 693.3 ± 536.8 x 10^6 SPZ/ml. Concentration increases towards the end of the season following a fall of the ejaculate volume. Mosaferi et al. (2006) reported for the bacterian camel a concentration equal to 414.8 ± 25.0 x 10^6 SPZ/ml.

At time 0 (before dilution), percentage of live spermatozoa in ejaculate averaged 60.9%. Hassan et al. (1995) reported only 40% of spermatozoa were found in live in an ejaculate of dromedary.This parameter was not affected by storage (20°C or 4°C) temperature but varied between used extender and time conservation (Table 2).

Table 2: Effects of extender on the proportion of live spermatozoa during storage (combined data of refrigeration and room temperatures).

Time conservation (h)	n*	Extender 0	Extender 3	Extender 4
3	38	57,8 ± 12,1	60,7 ± 12,4	55,1 ± 16,1
6	38	39,9 ± 21,9^{b}	55,3 ± 15,1^{a}	51,6 ± 14,6^{a}
24	38	36,0 ± 23,3^{b}	53,0 ± 17,9^{a}	44,2 ± 16,5^{ab}
48	38	10,3 ± 17,8^{b}	26,8 ± 24,6^{a}	23,7 ± 20,5^{a}

^{ab}: In each row, different subscript letter indicate significant difference (P<0.05).

After 3 h from collection, the proportion of live spermatozoa still high (>55%) and was comparable (P>0.05) for the three studied short term preservation extenders (D0, D3 and D4). After 6 h, the proportion of live spermatozoa in D0 declined sharply (P<0.05). However, there was a gradual decline in viability of the spermatozoa in the two other extenders and a significant(P<0.05) decrease was observed only after 24 h and 48 h conservation period for D4 and D3, respectively (Figure 1).

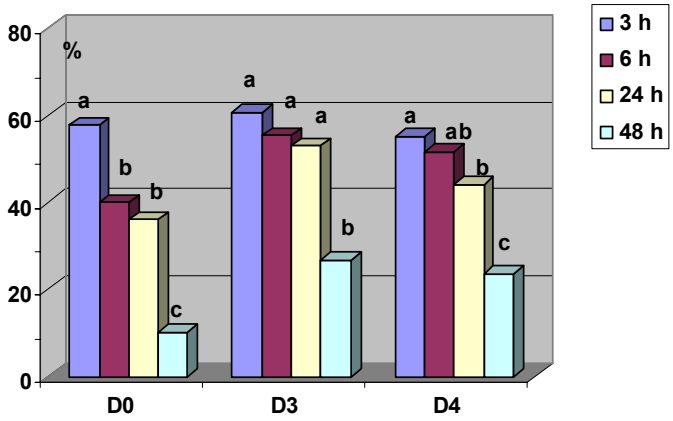

Figure 1: Effects of time storage on the proportion of live spermatozoa using a short term conservation extenders (D0, D3 and D4).
[abc] In each extender, different subscript letter indicate significant difference (P<0.05).

In this study, half skimmed milk show to be a good extender for short term conservation semen suggesting that lactose is suitable for spermatozoa of camel. One tris based diluent containing lactose was reported appropriate for camel semen but another extender without lactose let less proportion of motile spermatozoa (Wani et al., 2007). In fact, Tris-based extenders have been reported to be superior to many other diluents in preserving the motility of spermatozoa from red deer (Asher et al., 2000). This could be attributed to the fact that Tris has superior buffering qualities when compared with citrate and phosphate (Watson, 1979), and this is important, as spermatozoa are very sensitive to changes in pH (Levis, 2000).

Effects of extenders and thawing temperature-time pair on proportion of viable spermatozoa are summarized in Table 3. Percentages of live spermatozoa reduced after thawing in all freezing media The four tested temperature-time pair were comparable (P>0.05). However, there was a significant (P<0.05) difference between the extender on the proportion of viable sperm, it was lowest for D0 compared to other diluents (D1, D2).

Table 3: Effects of extenders and thawing temperature-time pair on proportion of viable spermatozoa.

Temperature-time pair	n	Extender 0	Extender 1	Extender 2
(55°C, 6")	21	18,9 ± 14,5 [b]	31,3 ± 12,3[a]	35,9 ± 12,6[a]
(55°C, 8")	23	18,5 ± 16,2 [b]	29,4 ± 13,0[a]	30,4 ± 9,9 [a]
(55°C, 12")	21	15,4 ± 11,9 [b]	30,4 ± 10,5[a]	35,3 ± 0,1 [a]
(40°C, 40")	22	15,0 ± 13,1 [b]	27,1 ± 10,1[a]	31,3 ± 9,7 [a]
Total	87	16,9 ± 13,9 [b]	30,0 ± 11,5[a]	33,1 ± 10,4[a]

[ab]: In each row, different subscript letter indicate significant difference (P<0.05).

In conclusion, semen short and/or cryopreservation of dromedary camel are feasible which could help the development of an immediate or differed artificial insemination in this species.

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Estradiol and testosterone profiles, their correlation with sexual libido in male camels

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Introduction

It has been observed that male camel is sexually active during a particular part of the year. It becomes refractory to sex at a particular stage after breeding season. Refractoriness to copulate in artificial vagina for artificial collection of semen has been an identified problem with some camels. Lack of libido is another identified problem in male camels. Testosterone (T) and estradiol (E_2) might be important in determining the libido and sexual behavior. The studies were undertaken to monitor estradiol and testosterone concentration round the clock at hourly interval once in a month over pre, during and post breeding season. Semen was also artificially collected and copulation time in artificial vagina and volume of semen were recorded. The hormonal profiles of corresponding months were correlated with copulation time and volume of semen ejaculated as an indicators of sexual libido. Because of the varied behavior of male camels in response to suitable sexual stimuli, eagerness or reluctance to ejaculate in an artificial vagina was correlated with circulating T and E_2 profiles.

Material and Methods

The study was conducted at National Research Centre on Camel, Bikaner using 6 adult Jaisalmeri male camels (*Camelus dromedarius*). E_2 and T profiles were monitored over pre, during and post breeding season using liquid scintillation Radioimmunoassay. Artificial collection of semen was attempted using standard artificial vagina (AV) method. Copulation time and volume of semen ejaculate were used as indicators of sexual libido. Logarithmic chart was prepared using hormonal profiles of E_2 and T and copulation time and volume of semen to correlate the endocrine profiles with sexual libido. Analysis of variance and paired t test were used to compare hormone profiles over different months and between sexually eager and reluctant to copulate in AV.

Table 1: Hormonal (T and E_2) Profiles and Sexual Libido (Copulation time and volume of semen) over different months in dromedary camels.

Months	Dec	Jan	Feb	Mar	Apr	May	Jun
Estradiol (pg/ml)	57.05 ± 1.91	86.69 ± 2.69	241.13 ± 12.27	238.93 ± 29.21	137.45 ± 14.98	114.27 ± 22.04	190.59 ± 49.21
Testosterone (ng/ml)	9.6 ± 0.4	22.1 ± 0.9	23.7 ± 2.2	10.9 ± 0.8	8.5 ± 0.8	2.2 ± 0.1	0.9 ± 0.05
Copulation time (Seconds)	216.8 ± 22.74	272.51 ± 20.90	300.13 ± 22.86	395.95 ± 42.42	320.32 ± 30.71	245.85 ± 31.41	Libido declined
Volume of semen (ml)	1.7 ± 0.29	2.13 ± 0.29	2.5 ± 0.28	4.76 ± 0.98	3.78 ± 0.66	2.76 ± 0.30	Libido declined

Results and Discussion

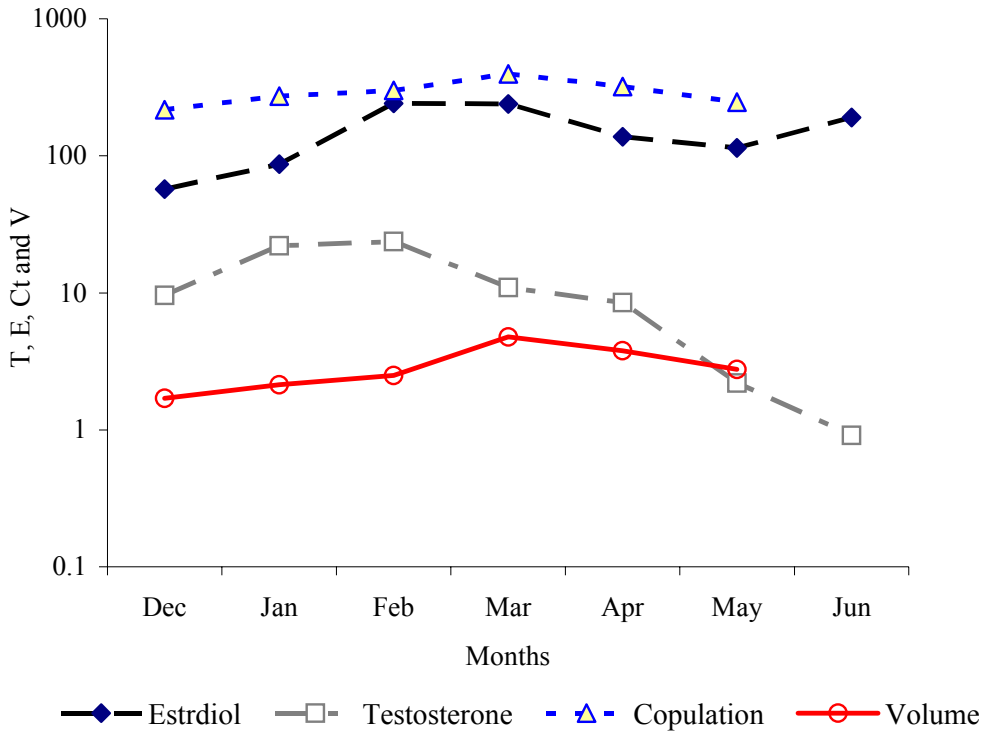
Hormonal profiles and parameters of sexual libido of 6 male camels over different months have been presented in Table 1. These data showed that T profiles were greatest during January- February, moderately high during December, March and April and low during May and June. The testosterone profiles during January-February were significantly high ($P<0.01$) from other 2 groups of months. Similarly T Profiles were also significantly high ($P<0.01$) during December, March and April from those during May-June. These data indicated an endocrine surge of testosterone during January-February, which declines considerably over March-April and reach minimal basal levels during May and June. Individual variations were observed in duration of endocrine surge. Cessation of endocrine surge was evident by low basal levels of T during March, April and May in 1, 2 and 3 of six animals, respectively.

Estradiol concentration was also greatest during February followed by March. Estradiol concentration during February- March was significantly greater ($P<0.01$) than other months. Estradiol concentration was the lowest during December and January months. Estradiol concentration declined during April and May. It increased again during June.

Copulation time in AV used as an indirect indicator of sexual libido was greatest during March followed by April and February. The sexual libido declines during May and lost in ensuing months. Sexual libido regains from December to reach to its peak during February- March and April. Exactly similar trends were noted in another indirect indicator of sexual libido i.e. Volume of the semen ejaculated.

Fig. 1 shows a logarithmic chart showing correlation between hormonal profiles and sexual libido. It appears that sexual libido is manifested in camel due to increased concentration of T and E_2. With the decline in concentration of T in particular, the sexual libido declines slowly to the levels of reluctance to sex.

Figure 1 Correlation of hormonal profiles with sexual libido



For a group of 4 males who had effectively copulated and donated semen in artificial vagina and a male who was refractory to mount and copulate in artificial vagina but chased and covered when let loose with females, data on T and E_2 concentration have been presented in Table 2. The data revealed that T profiles during December, May and June were significantly higher ($P<0.01$) in male refractory to copulate in AV than those, which adequately copulated while the differences in testosterone concentration during January, February, March and April between 2 groups were not significant ($P>0.05$). The data also showed that estradiol concentration in refractory male was significantly higher ($P<0.01$) during December and January than other group while the differences were not significant ($P>0.05$) during February, March, April, May and June. The data indicated that the psychic inhibition to copulate in artificial vagina was not due to

deficiency of T and E_2. Studies on the sexual behavior in laboratory animals have indicated roles of neuro-transmitters in regulation of sexual desire (Hull et al, 1999). Similar studies in camels have not been reported but it can be speculated that differences in sexual behavior of camels as observed in present study may be due to altered neuro-transmitters release from centers located in brain.

Table 2: Estradiol and testosterone profiles of a group of 4 male camels compared with a psychic impotent male camel

		Dec	Jan	Feb	Mar	Apr	May	Jun
Estradiol (pg/ml)	Potent males	54.71 ± 2.00	89.37± 2.88	203.37± 11.10	243.20± 41.05	141.74± 21.50	121.04± 32.57	187.93± 63.40
	Psychic impotent male	81.14 ±* 3.42	107.84 ±* 5.59	214.32± 9.85	251.62± 52.46	111.89± 23.85	135.47± 18.82	56.96± 18.39
Testo-sterone (ng/ml)	Potent males	8.40± 0.46	26.12± 0.82	20.68± 1.02	9.38± 1.20	8.86± 1.20	1.90± 0.17	0.68± 0.04
	Psychic impotent male	14.80 ±* 1.06	24.03± 1.93	22.28± 2.13	9.27± 0.83	4.89± 0.78	3.43±* 0.43	1.11±* 0.12

*Statistically significant

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Optimization of embryo recovery in the dromedary camel (Camelus dromedarius) : The bull factor

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Introduction

Embryo Transfer in the racing dromedary camel is a complex reproductive manipulation of female donor and surrogate camels involving a number of potentially limiting steps, any one of which can greatly influence the success of the procedure. Under normal circumstances adult female camels have a gestation period averaging 13 months and as such only have a calf every two years. An embryo transfer program can allow a single donor female to produce up to 15 calves from multiple bulls in a single season.

The female camel is an induced ovulator and as such needs the stimulation of mating and the presence of seminal fluid to allow ovulation to occur. Success with embryo recovery depends on the adequate stimulation of the female ovary to produce multiple follicles and the ability of the bull to stimulate ovulation and fertilise the ovum with motile sperm. While artificial insemination is a valuable tool in producing pregnancy in a female camel on heat it has very poor results when it is combined with superovulation and attempts to recover multiple embryos from donor females (McKinnon et al 1994).

When trying to optimise embryo recovery in an embryo transfer program often too much emphasis is made on the female side of the equation with regards to selection of female, her history of superovulation, time of flushing, flush technique with the choice of the males and their previous performances often overlooked.

Bull Selection

Young bulls are selected primarily on race track performance times and as such it is rare to see a bull come into the programs until it is at least 7 years of age, unless it has been injured early in its career. Earlier work at the centre has shown that sperm counts generally don't reach optimum till at least 5yrs of age and so even an injured champion would not be used before this age. Older bulls often show a decrease in

sperm quality after 20yrs (Manefield and Tinson 1996). With older bulls the progeny testing side of performance becomes more important for the owners and now that robots are being used to race, performance can be assessed in offspring as young as 12 months of age (Tinson et al 2007). One particularly famous old bull, Saraab is so highly regarded that there is a permanent camp of female camels and owners waiting outside his pen for the chance of a mating, often decided by ballot. A female offspring to him can be worth up to $50,000 US as a new born calf. The male's race history including 6-8 km time records, number of cars won and possible gold cup triumphs all heavily influence the balance when selecting a stud bull. History records on better performed offspring can heavily change the potential use of males from season to season.

Management of Bulls

Bulls are housed individually with the females generally brought to the males when ultrasound (Tinson and McKinnon 1992) determines the size of multiple superovulated follicles optimum (10-16mm). The bulls generally receive fresh lucerne, concentrates, dried hay as well as milk and dates. Optimally they are rested for a minimum of 7 days prior to mating their first E.T. female and are rotated one day on and one off depending on the availability of other bulls and the number of females in the program.

Season is important in regards to bull's libido. While natural matings are carried out as early as September and as late as May we generally restrict the embryo transfer females to matings between the first week of November and the last week of March. While libido is reported to be improved in male camels with the use of GnRH out of season (Moslah et al 1992), we have not used this technique on our stud bulls. Fitness of the bull in terms of length of mating and the amount of vigour per mating also had a role to play. Some very old bulls have been known to sit very quietly on the female, ejaculating but resulting in low or negative ovulations and thus poor ovulation rates (pers. obs.). Females with a mating of a shorter duration than 5 minutes would generally receive an injection of GnRH (Receptal, Hoechst).

With regards to the health management of the bulls during the season, all bulls receive a full check up prior to the breeding season including full blood count and routine worming as well as being checked for trypanosomes. Historically we used to do routine semen counts, but these were often difficult to assess due to the thick pre-seminal fluid present. In recent times, partly due to time constraints and the huge value

assigned to many of the bulls we don't perform counts unless a major problem is suspected. New techniques developed in Iran (Niasari 2007) have greatly improved the ability to more accurately assess semen quality.

Semen Quality

In the late 80's and early 90's we performed numerous analyses of semen via electoejaculation (under general anaesthesia) and found a large range in semen quality of dromedary bull camels (McKinnon and Tinson 1989). Variations in volumes of ejaculate from 3-8 ml, with concentrations ranging from 100-800 million/ml were common. Overall semen motility varied considerably from 50-90% with lows of 10% progressive motility to highs of 40% (Manefield and Tinson 1996). Depending how these figures combined in individual bulls the total sperm counts could vary from 500 million to highs of 7 billion. This coupled with motility variations could obviously affect the potential outcome in terms of fertilisation of multiply ovulated ovum.

Strike Rate

While track performance is the ultimate determinant of mating regimes in our breeding programs we do have the luxury of a large stable of very well performed bulls to choose from. Many of the bulls are either Gold Cup winners or older bulls who themselves have produced offspring with superior performance. When the preparation of the females is optimum with 20 plus follicles ready to be ovulated it can often be frustrating to find consistently low embryo recovery from the use of certain bulls.

Semen quality undoubtedly plays a huge role in this outcome, the high value (often many millions of dollars) and demand for these stud bulls mean regular semen analysis is difficult if not impossible to achieve. There is little opportunity to train these animals to an artificial vagina and electroejaculation even under anaesthesia creates potential medical, ethical and stress issues (particularly on the veterinarian).

To counter this we have looked at trying to rate bulls on their average ability to fertilise ovum from detected ovulations post mating. We have borrowed the term "strike rate" from the cricketing world and rated the bulls on a percentage based on the average number of embryos recovered from female superovulated camels per ovulated follicle. There is no doubt that being 100% accurate in counting the follicles in a superovulated female can be difficult, especially when follicle numbers approach 15-20 per ovary. Counting residual follicles at 72hrs is more accurate when calculating total

ovulations. Consistently poor results were seen with Shaheen Khaili where his strike rate was only 25% over 18 matings. Two particular mating resulted in the recovery of 4 embryos (E's) and 9 unfertilised eggs (UFO's) from 25 follicles and 1 E and 4 UFO's in a mating with 13 follicles. Large numbers of UFO's always lead one to question semen quality. Maschour on the other hand delivered a rate of 50% with his best effort being 29 embryos from detected presence of 28 follicles with 26 ovulations (obviously there must have actually been at least 31 follicles in reality).

Table 1. Bulls used in seasons 2006/7 and 2007/8 showing age, numbers of females

BULLS NAME	AGE	MATINGS	FOLLICLES	OVULATIONS	EMBRYOS	STRIKE RATE
SHAHEEN KHILAILI	20	18	326	301	74	25%
SARAAB	25	16	304	268	63	23.50%
SARAJ	12	5	68	65	34	52%
MILEH SAGEER	16	2	54	48	12	25%
DHAB	12	7	99	93	30	32%
MOBTA	7	9	154	149	36	24%
NASHWAN	8	9	140	133	51	38%
MASHCOUR	7	7	164	148	74	50%
SHAHEEN NAYHAN	10	2	40	39	14	36%
SHAHEEN GAITH	9	8	104	96	41	43%
SADEH	20	4	51	48	1	2%

mated, detected number of follicles and ovulations, embryo recovery and "strike rate" (embryos recovered per ovulation expressed as a %).

Summary

Managing an embryo program with high value female racing camels is a meticulous and time-consuming procedure. Having optimised the female preparation it is essential to ensure that the bull camel used gives the best chance of good embryo recovery numbers and thus greater opportunity of multiple individual pregnancies in surrogate mothers.

A newly retired female race camel producing in the order of 25-30 follicles has the potential to recover 6-8 extra embryos if given access to a bull with a strike rate of 50% vs. 25%. Given an average transfer pregnancy rate of 65% this could translate to 4-6 extra offspring. The recent high prices of not just racing offspring (2-3 million US)

but “beauty contest” camels (up to 5 million US) makes these bull considerations very important when deciding mating match ups.

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In vitro embryo production in camelids: An overview

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Introduction

In vitro embryo production (IEVP) technology has been used to produce large number of embryos for transfer and for manipulations in a number of animal species. However, basic manipulations such as oocyte collection and their maturation, collection and preparation of semen for IVF procedures, culture and passaging of cells for SCNT, oocyte/cytoplast activation, culture media and conditions for embryo culture are some of the factors affecting the IVEP technology. Also, the interspecies differences in the maturation physiology do not allow the direct extrapolation of IEVP system from one species to another. In camelids, relatively limited information is available on development of this technology. The objective of the current presentation is to give an overview of the present status of in vitro embryo production in camelids.

Collection of cumulus oocyte complexes (COCs)

Ovaries collected from the slaughterhouse are usually processed as quickly as possible, however, storing them at room temperature for up to 12h does not seem to have any detrimental affects on the maturation rate of oocytes. (Wani and Nowshari, 2005). The COCs have been collected by follicular aspiration, ovarian slicing, mincing of ovaries or by excising the follicles and teasing them apart under a steriozoom microscope. A higher recovery rate of oocytes was achieved with the later method when compared with aspiration with a syringe and needle or an aspiration pump (Nowshari, 2005). Similarly, slicing of the dromedary ovaries was found to be effective compared with aspiration for collection of oocytes (Abdoon, 2001). In llamas, mincing the ovaries by a razor blade (Del Campo et al., 1994) yielded more oocytes per female than aspiration of follicles (Del Campo et al., 1992).

Ultrasound guided trans-vaginal ovum pick up (OPU) up has been used in llamas, alpacas and camels to aspirate the follicles from stimulated or non-stimulated ovaries. In llamas, ovarian superstimulation with eCG was associated with a slightly higher proportion of expanded COCs and oocytes in metaphase II, compared to superstimulation with FSH (Ratto et al, 2005) whereas, in dromedary camels, eCG and

FSH used together gives better results for superstimulation of the ovarian follicles. The best response to gonadotropins is obtained when treatment is initiated after elimination of the dominant follicle or synchronization of follicular waves.

In vitro maturation of oocytes

The majority of oocytes collected from slaughterhouse ovaries are in germinal vesicle stage, but at 20h of culture most of them had undergone germinal vesicle breakdown (Wani and Nowshari, 2005). In the initial studies on the maturation of dromedary camel oocytes a maturation time of 36h was considered to be optimum. However, studies on kinetics and ultra structure of oocytes during nuclear maturation suggest the optimal culture time to be around 30- 32 h (Wani and Nowshari, 2005; Kafi et al., 2005). Also, the proportion of blastocysts obtained from the oocytes activated after 28 h of maturation were higher when compared with oocytes activated after that period of maturation in a recent study (Wani, 2008a). Abnormal chromatin configuration and degenerative changes have been observed after 40h of in vitro culture in camel oocytes. The COCs of llamas have also been initially cultured for 32–36h (Del Campo et al., 1994) but a recent study has shown that an incubation time of 28-30h resulted in higher maturation rates (Ratto et al., 2005). In Bactrian camel, 46.7% of oocytes achieved meiotic maturation after 24–26h of culture (Shorgan and Pang, 1993). Ultra structural studies during the maturation process of camel oocytes showed an increase in the perivitalline space of oocytes as the maturation process progressed until 24h, but no further increase occurred until 36h of culture (Kafi et al., 2005).

Tissue culture madiun-199 (TCM-199) is mainly being used for IVM of camelid oocytes; however, Ham's F10 (Kafi et al., 2005) and CRIaa medium (Abdoon, 2001) have also been used in dromedary camels. In a study, TCM-199 was found superior to CR1aa or modified Connaught Medical Research Laboratories medium-1066 for dromedary oocyte maturation (Nowshari, 2005). No difference has been observed in the proportion of oocytes reaching M-II stage between the media supplemented with fetal calf serum, estrous camel serum or bovine serum albumin, however, a supplementation of 20 ng/mL of EGF to the maturation medium increased the oocyte maturation rate when compared with the media supplemented with 10 ng/mL, 50 ng/mL or no EGF groups (Wani and Skidmore, 2008).

Llama oocytes obtained by surgical aspiration 22h after buserelin administration had a maturation rate of 62% in the absence of hormones (Miragaya et al., 2002), while in

alpacas, a maturation rates of 40–46% were obtained when COCs collected 18–24h after hCG administration were incubated for 26h (Gomez et al., 2002). Treatment with LH after ovarian superstimulation in Llama (Ratto et al., 2005) and GnRH in dromedary camels (unpublished data) permitted the recovery of expanded COCs most of which were in M-II stage.

In vitro fertilization and intracytoplasmic sperm injection

The production of embryos by IVM/IVF in camelids was first reported in llamas (Del Campo et al., 1994); however, the first offspring's were produced recently in dromedary camel (Khatir et al., 2006). In vitro production of camel embryos has been reported using fresh ejaculated (Khatir et al., 2006) and stored epididymal semen (Wani, 2008b) with a cleavage rate of 64% and 43-60% and a blastocyst production rate of 36% and 12-24%, respectively. In llamas, 32% oocytes cleaved after IVF with epididymal semen while the percentage of embryos reaching morula, early blastocyst and hatched blastocyst stages were 5.6%, 6% and 4.7%, respectively (Del Campo et al., 1994). All the above studies have shown that the chronology of embryo development in camelids is faster than in other species irrespective of the source of spermatozoa used in IVF.

Intracytoplasmic sperm injection of llama oocytes using ejaculated spermatozoa resulted into production of 16% morula stage embryos but no blastocysts were obtained (Miragaya et al., 2003). In dromedary camel, epididymal spermatozoa were injected into the IVM oocytes of abattoir origin and blastocyst production rate was similar to that of IVF (unpublished data).

Chemical activation and nuclear transfer

Recently a protocol for activation of camelid oocytes has been optimized (Wani, 2008a) in which, in vitro matured dromedary oocytes were activated with 5 μM ionomycine or 7% ethanol followed by exposure to 6-diethylaminopurine or roscovitine. It has been shown that activation of oocytes, after 28 h of maturation, with 5μM ionomycine for 3 min and subsequent culture in 6-DMAP for 4 h, gives optimal results and higher blastocyst production rates. Production of embryos by NT has been reported in llamas (Sansinena et al., 2003), in which adult fibroblast cells were used as the nuclear donors with a fusion rate of 62.5%, followed by cleavage rates of 32 and

40% in CR1aa and G1.2 medium, respectively. However, transfer of embryos surgically into the oviduct or non-surgically into the uterus did not result in any pregnancies. In preliminary studies on dromedary oocytes, a fusion rate of 70-80% was observed after enucleated zona free oocytes were fused with granulose cells, but a low blastocyst production rate was observed. No embryos were transferred to recipients in this experiment (Wani et al., unpublished data).

Conclusion

In vitro embryo production is not very efficient in camelids when compared with other domestic animal species but increased research activity and publications in recent years on developing this technique are promising. However, a very low availability of the slaughterhouse material in these species is the limiting factor for the development of these techniques. The ability to store ovaries for up to 12 h without a deleterious affect on in vitro oocyte maturation can be of tremendous help for their transportation over long distances, but more studies need to be concentrated on the collection of oocytes from live animals by ultrasound guided ovum pick up (OPU) like cattle or other domestic animal species.

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Effect of the day of uterine flushing on embryo recovery rate in superovulated alpacas (Lama pacos)

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Introduction

Alpaca breeding is an important economic activity in the Peruvian Andes, but it mainly occurs under a traditional production system of small herders. Thus, breeding practices have led to a significant decrease of genetic quality of alpacas in Peru (Fernández Baca, 1993). As a consequence, development of technologies to genetically improve the alpaca and its reproduction is necessary. Genetic progress in cattle has been enhanced by the use of reproductive biotechnologies as Embryo Transfer (McDaniel and Cassell 1981), so that it could be useful in alpacas. In order to take better advantage of ET it is required successful superovulation protocols and optimal embryo recovery rates.

Superovulation in alpacas has been achieved but embryo recovery rates following uterine flushing on days 7 or 8 post mating is low (Huanca 2005). This could be due to different speeds of development (Novoa et al 1999, Bravo et al 2004), or oviducal transport of the alpaca embryo as compared with other species. . The objective of this study was to evaluate the embryo recovery rate following uterine lavage on either day 5, 6 or 7 post mating.

Material and Methods

A total of 21 adult alpacas with normal parturition history and/or ≥ 15 day-post-partum period were used and their uteri and ovaries evaluated by transrectal ultrasonography. Ovulation was induced in alpacas with a dominant ovarian follicle ≥ 7mm with a single i.m. injection of GnRH (0.004 mg buserelin) and after two days were superovulated with a single i.m. dose of 650 IU eCG. Five days after eCG administration a single dose of prostaglandin (0.25 mg cloprostenol) was injected i.m. and the next day alpacas were evaluated by transrectal ultrasonography and the number of follicles recorded before they were mated, alpacas at this time had ovarian follicles ≥

6mm. After mating, they were distributed in 3 groups: G1 (embryo recovery at day 5), G2 (embryo recovery at day 6), G3 (embryo recovery at day 7). Day 0 was considered as the day of mating. Additional ultrasound evaluations were carried out on the day of embryo recovery to register the presence, number and size of the corpus lutea. The uterus of each alpaca was flushed non-surgically using a two-way Foley catheter and 250 ml of embryo flushing media per uterine horn. The number, size and developmental stage of all the embryos recovered were recorded.

Results

Number of alpaca embryos recovered was not significantly different between groups G2 and G3, but there was a greater number for group G2 (4.3 ± 2.4 embryos/alpaca) than for group G3 (3.0 ± 2.9 embryos/alpaca). No embryos were recovered in group G1. Moreover a bigger size of embryos was registered in G3 (0.47 ± 0.22mm) than in G2 (0.25± 0.05mm). Embryos recovered were mainly at hatched blastocyst stage. There were no significant differences ($p<0.05$) among groups in the number (G1=10.7± 2.1, G2= 9.6 ± 2.4, G3= 10.0 ± 3.3) and size (G1=8.3± 1.6mm, G2= 8.5 ± 21.8mm, G3= 9.6 ± 2.6mm) of follicles; and the number (G1= 9.6 ± 2.2, G2= 8.7 ± 2.1, G3= 8.6 ± 3.0) and size (G1= 8.9 ± 1.77mm, G2= 8.9 ± 1.3mm, G3= 8.8 ± 1.2mm) of corpus luteum.

Discussion

Results obtained in this study, related to embryo recovery rate by non-surgically uterine flushing present significative diferences ($p<0.05$) among day 6 (G2) and 7 (G3) respect to day 5 (G1).

Results indicate that embryo development in superovulated alpacas appeared to be faster than in other species, finding hatching blastocyst stage since day 6 post mating, while in cows blastocyst leave the pellucid zone at day 9 or 10 (Massip et al 1982). As a consequence that embryos do not have a pellucid zone might mean that they have more susceptibility of being damaged during non-surgically uterine flushing, and it could be affecting the number of embryo recovered. In fact, the number of corpus lutea show low correlation with the number of alpaca embryos recovered, differentially to correlation found in cows (Kanuya et al. 1997).

Size of embryos present significative differences ($p<0.05$) between G2 (0.248 ± 0.052 mm) and G3 (0.470 ± 0.220 mm). Some reports in other species suggest that older

and bigger embryos could be more easily damaged than younger and smaller embryos, when they are transferred by non-surgically methods and it could be a possible reason for lower pregnancy rates (Iuliano et al., 1985).

Conclusions

These results suggest that the alpaca embryo does not enter the uterus until day 6 after mating and it develops very rapidly as it appeared to almost double in diameter from day 6 to 7.

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Sex determination of alpacas by amplification of the SRY (Sex-determining Region Y-chromosome) gene

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Abstract

A method for sexing Alpacas DNA has been developed using amplification of the SRY (Sex determining region Y-chromosome) gene. Oligonucleotide primers were designed according to the SRY gene partial sequence from Male Guanaco (*Lama guanicoe*). After electrophoresis, a normal male alpaca showed a single SRY band of 146 bp that did not appear in female alpaca samples. After optimization, the PCR procedure for sex diagnosis was applied to 10 DNA samples of alpaca. The sex as diagnosed by PCR corresponded with anatomical sex determination in all cases. This study demonstrates that the present method can be applied to the DNA samples sexing from Alpacas by PCR amplification SRY gene.

Introduction

Alpaca farmers are looking for increase the population of their herds, keeping the females permanently and selecting the best males for the breeding season. One male embryo of Alpaca can be more expensive than female, if is progeny of a recognized male winner of morphological contest. Therefore with sex identification of embryo the aggregate value increases if is male. The selective breeding for desirable characteristics can be easily diffused using males with high level of heritability of characteristics to their progeny. The control of the sex in the newborns can be applied using sexed semen in fertile females or transfer sexed embryos (Seidel, 2003).

Limited information exists for a diagnostic technique that can be identified the sex of Alpacas DNA. Some studies have shown that molecular based methods can be used for identification of sex by amplification of specific segment of the sexual chromosome. The SRY gene which is only present in the Y chromosome (Koopman et al.,1990) was applied for sexing DNA samples of Llamas (*Lama glama*) (Drew et al., 1999).The aim of the present study is to adapt the same technique in Alpaca sexing.

Materials and Methods

Isolation of Genomic DNA from lymphocytes: Blood samples were collected from a group of 10 Alpacas (5 per each sex). All samples was processed using the Maxwell® 16 DNA Purification Kit (Promega Corporation).

Olygonucleotide Primers design: Using the interactive web-based program for designing primers (GeneFisher), a pair of primers was designed to amplify the SRY gene using the partial sequence of the Guanaco SRY gene (GenBank accession U66068).

PCR Protocol: A final volume of 100 µl of Master Mix per each reaction was prepared containing: 10 µl of PCR buffer [10X / µl], 10 µl MgCl2 [25 mM / µl], 2 µl dNTP mix [10 mM / µl], 2 µl each primer [50 pM / µl] µ 65.2 µl of water PCR, 0.8 µl Taq DNA polymerase [5 U / µl] and 8 µl genomic DNA per each animal. PCR tubes were placed in a Thermal cycler DNA Engine (BIORAD., Mexico) with the following thermal program 94 °C for 5 minutes, followed by 35 cycles of 94 °C for 45 seconds, 58 °C for 45 seconds and 72 °C for 1 minute with final step of 72 °C for 10 minutes. The PCR products were analyzed by electrophoresis using agarose gel 2% stained with ethidium bromide and viewed under ultraviolet light. The picture of the gel was taken by the system UV trasluminator with digital camera (BIORAD, Italy). Sequencing of the amplified purified PCR product of the SRY gene was was out sourced to AGOWA, Germany.

Results and Discussion

The sequence for the Set of Primers was: Forward: 5'- TGT CAT ATG TCG TGA GGC -3' and Reverse: 5'- TTC GAG GAG GCA CAG AG -3'.

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> 1 gtcaagcgcc ccatgaatgc tttcattgta tgggctcgtg atcaaaggcg aaaggtggct
 61 ctagagaatc ccaaaatgca gaactcagag atcagcaagc ggctgggata ccagtggaaa
121 ttgcttacag aagctgaaaa gcggccgttc ttcgaggagg cacagagact acg
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Figure 1. SRY gene nucleotide sequence of Guanaco (GenBank U66068). The sequences homologous to the primers achieved are underlined.

The SRY gene amplification was tested using 10 DNA samples from alpacas with anatomical known sex. Figure 2 shows the presence of an exclusive band to male alpacas. All PCR sex determinations were identical to those from anatomical examination of the same animals sampled.

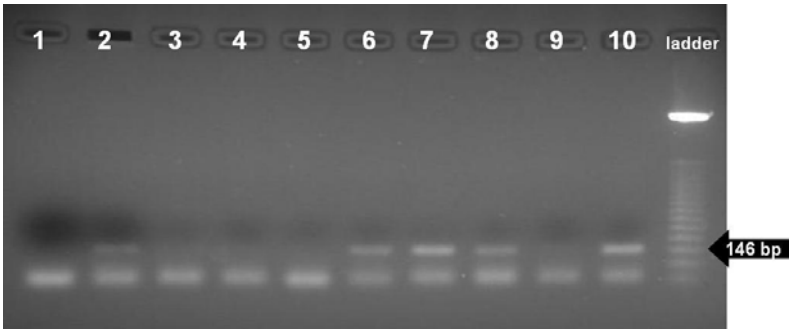

Figure 2. PCR SRY gene in DNA from Alpacas. 2, 6, 7, 8 and 10: Alpacas male (XY) SRY positive. 1, 3, 4, 5 and 9 Alpacas female (XX) SRY negative.

The sequencing of the PCR product from the Alpaca SRY gene revealed a sequence of 146 base pairs with 100% sequence homology with partial sequence of Guanaco SRY (GenBank U660668). The new sequence was submitted in the GenBank with the code DQ862123 and the definition "Lama pacos sex-determining region Y gene, partial cds." The results of this experiment show the feasibility of the Alpaca DNA sex identification of the Alpaca using blood samples and suggest the hypothesis of the feasibility of identifying sex in samples of semen or pre-implantational embryos. Achieving Alpaca with the desired sex, contribute to the technologies of artificial insemination and embryo transfer in Alpacas.

Conclusions

The PCR technique for sex identification of Alpaca from DNA samples was achieved by amplifying partially the Alpaca SRY gene (DQ862123).

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Sex determination of the 7-day camel embryo before transfer

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Introduction

Many sectors of the camel industry prefer a preponderance of animals of one sex. In the racing industry of the Middle East, female camels are the preferred racing animal often fetching prices in excess of $1 million US, while a rise in interest in camel's milk will see the possibility of sex selection being incorporated in breeding programs for milking animals. The primary sex of camelid species, as in the overwhelming majority of mammalian species, is determined by the presence or absence of the entire Y chromosome or a functional portion thereof. The essential portion is a gene known as SRY that is responsible for initiating testis differentiation (Sinclair et al 1990). Analysis of DNA sequences from an embryo biopsy on this portion associated uniquely with the Y chromosome will allow predetermination of the sex of the embryo.

Materials and Methods

In March 1998 nine female retired racing camels between 6 to 12 years were prepared to be donor females along with 35 recipient surrogates. The camels were prepared as previously described (McKinnon et al 1994) with the donors being flushed at 7 days post mating. Four of the donors had a good response producing 23 embryos (17 hatched blastocysts and 6 expanded blastocysts) in total.

Recovered embryos were first placed in Vigro ABTech Holding solution and then placed in Virgo Splitting Solution under oil to allow micromanipulation to take place. A Transferman Micromanipulation System (Eppendorf Germany) attached to a Leica inverted microscope was used to visualise the embryos. The 23 embryos were divided into 2 groups with 17 receiving a small biopsy of an estimated 5-20 cells from the outer trophoblastic layer of the embryo with the remaining 6 embryos initially split into demi-embryos (in the hope of producing twins) and also biopsied at the same time. The biopsies were performed by attaching an AB Tech Twinning blade to the Eppendorf

Transferman robotic arm and manipulating it by joy stick while the embryo was held with electrostatic attraction to the bottom of the dish in the splitting media.

The biopsied embryo is retrieved and placed in holding solution while the biopsy was transferred to a 20ul Corbett research thermocycling capillary containing 2 ul of lysis buffer. The biopsy was then incubated and placed in ice and then added with 16 ul of a duplex PCR master mix including male specific primers based on a patented male specific polynucleotide sequence and camelid specific autosomal control primers. The capillaries were then placed in a Corbett Research FTS-400 Capillary Thermocycler and subjected to 60 cycles varying from 72 to 95 deg C. The contents were loaded on to a 2% agarose gel. The ethidium bromide stained gel was then visualised under UV radiation. No bands are observed in the absence of camelid DNA, where as a single 230 base pair band is present in the presence of both male and female DNA (internal autosomal control) and if male DNA is present there is another 470 base pair band present.

More recently the protocol was adapted to use with a real time PCR format using the Corbett Rotogene instrument. This system uses the male specific and autosomal primers and probes with advanced centrifugal, real time DNA amplification. The rotary nature of the machine allowed shorter holding times in the PCR ramping with temperatures between 25-99 deg C. The machine has 4 separate light sources and 6 separate detection filters. The rotogene software integrates the PCR machine and a Hewett Packard computer to give a read out of the detection of the Y specific sequences as well as the autosomal DNA. Three embryos (expanded blastocysts) were collected in January 2000 and biopsied and transferred after analysis with the Rotogene to test the system.

Results

Biopsies were performed and analysed with 16 of the embryos being female and 7 male. Nineteen of the biopsied embryos were transferred to surrogates with the establishment of 9 pregnancies, which continued to term with the world's first pre-sexed embryo transfer calf born on 3/4/99 with the predicted female and a gestation of 360 days. The last of the presexed embryo births were born on the 24/4/99 with a gestation of 381 days. The Jan 2000 sexing results gave 2 female and one male embryo and the transfers resulted in 2 pregnancies being born 390 days later. All births were within

normal gestation range for dromedary camels (Manefield and Tinson 1996, Tibary and Anouassi 1997).

Discussion

The sexing of the pregnant foetus in the camel at 60 days as is done in the horse (McKinnon 1998) is difficult in the camel. Also once the pregnancy has established the result is fixed where as with pre-sexed embryos a decision of which sex to transfer can be determined. This project work was in many ways a direct follow on from work done in ruminants by Reed and co-workers in the 1980's (Reed et al 1986).

In a diagnostic assay for genetic sex, no detectable product of PCR amplification from male-specific primers may result not from a female sample but from PCR failure or loss of sample. For this reason a duplex PCR assay in the Rotogene was developed in which a 200 base pair fragment of an autosomal repeated sequence was simplified simultaneously with the Y-specific target. The Rotogene is able to simultaneously detect both reactions to confirm a reliable test has been carried out. The Y-chromosomal specific element CY.AM11 has been shown to be present in all camelid species tested which included dromedary and Bactrian Camels, Llamas and Alpacas (Harrison 2001).

The pregnancy rates of 9/19 (47 %) and 2/3 (66%) compared well with other reported pregnancy rates in camels (McKinnon et al 1994, Tibary and Anouassi 1997, Tinson et al 1998). Electrostatic attraction of embryos to the floor of the dish doesn't allow the accurate positioning of embryos in regards to twin splitting of embryos and the recent use of a newer micropipette (Cook Australia) has allowed for better results with twinning and biopsies (Tinson et al 2001). The development of the Rotogene analysis allowed the ramping time to come down to less than 1 hour with larger biopsies being positive within 27 cycles (Harrison,2001).

Conclusion

The determination of the sex of the camel embryo is a viable procedure that gives results comparable to fresh embryo transfer without manipulation and offers the flexibility to use the embryos and surrogates more efficiently when doing embryo transfer in camels. This is particularly evident when selecting sex for racing or milking camels. The "sex test "looks set to be just the first in a series of tests that will be able to apply to the camel embryo. Just as a blood test is routine, a DNA screening much like

is already happening at human IVF clinics could be developed for camels. Conservation projects currently envisaged for the Wild Endangered Bactrian Camel in the Gobi Desert will also have the potential to benefit from the sexing of its embryos so that the female groups can be increased in number at a more rapid rate when applying assisted reproduction.

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Differentiation of six Pakistani camel breeds by molecular genetics analysis

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Introduction

Camel is a queer animal; an inhabitant of hot deserts and also cold mountains. Camel population in Asia is not genetically homogeneous rather it is a multitude of breeds adapted to diverse cultural, ecological and economic contexts. Genetics could be a powerful tool to modify animal growth rate and body composition, and has been largely used to adapt animal production to the market requirements (Guernec *et al*., 2003). In the present project an effort has been made to determine genetic analysis for genetic differences in the local camel breeds, which have so far been differentiated only on the basis of phenotypic appearance, geographical location or on the tribal ownership.

Materials and Methods

A total of 157 camels belonging to six different Pakistani breeds Viz. Marecha (n= 26), Dhatti (n= 24), Larri (n= 27), Kohi (n= 30), Campbelpuri (n= 25) and Sakrai (n= 25) were included in this study. These animals belonged to different ecological zones of Pakistan and presented a high variation in their coat colour.

Genomic DNA was isolated from their hairs using NucleoSpin Tissue kit (Macherey-Nagel, Germany) according to the manufacturer's protocol. For primer design sequences from the NCBI GenBank were used. PCR reactions were carried out using UNO thermo cycler (Biometra, Germany) in a total volume of 25 µl containing 2.5 mM, $MgCl_2$, 0.2 mM dNTP 1U Taq DNA Polymerase (Genaxxon, Germany) 0.2 µM of forward and reverse primer and 100 ng genomic DNA. After an initial denaturation with 94°C for 2 min 35 cycles were done each consisting with 94 °C for 1 min, 56 °C (primer pairs MN-1B up and MN-1B low) for 30 sec and 72 °C for 40 sec. The final step lasted for 10 min at 72 °C. PCR amplified fragments were excide from 2% agarose gel and purified using Gene Clean II Kit (Q BIO gene, Canada). The

fragment was sequenced in both directions using BigDye Terminator v1.1 Cycle Sequencing chemistry on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, USA). All sequence alignments and distance calculations were made by Lasergene software (DNAStar, USA).

Sequencing was carried out by ABI 310 Genetic Analyzer (Applied Biosystems, USA), whereas sequence alignments were made by DNA Star software. For sequence analysis, primer design and gene editing the software Lasergene (DNASTAR, USA) with its sub programmes "Megalign", "Primer Design" and "Editgene" were used, respectively. Multiple sequence alignment was carried out by Clustal W (1.82) using EBI Server.

PCR reactions for a Dde I restriction were carried out in a total volume of 25 µl with the primer pair D-TY-A up and D-TY-A low to amplify a 474 bp fragment at 56°C. PCR product (15 µl) was mixed with master mix comprising 2.0 µl buffer, 0.2 µl bovine serum albumin (BSA), 0.2 µl Dde I enzyme (2 U, Promega Madison WI, USA) and 2.6 µl water and incubated at 37°C for 3 hours. After incubation, 5 µl loading dye buffer was added and electrophoresed for 2 hours.

PCR reactions for *Msp* I analysis were carried out in the same way as for *Dde* I restriction but with D-GH 2 up and GH1-3A low primer pair 62 °C. Similarly for *Sfc* 1 restriction (Melanocortin 1 receptor gene) PCR reactions were carried out with MC-end up and MC- bend Clow primer pair at 61°C.

DNA Walking Up Premix kit (Seegene, USA) was composed of PCR Master Mix and unique DNA Walking ACP (DW-ACP) primers that were designed to capture unknown target sites with high specificity.

General linear model (GLM) was used for analysis of Variance (ANOVA). The Unequal N HSD test was applied to determine the significant differences between group means. Different genotypes were compared by Chi square test for each selected breed. Kruskal-Wallis test was used to compare restriction fragment length polymorphism (RFLP) results and all the phenotypic parameters studied.

Results and Discussion

Melanocortin 1 Receptor (MC1R) Gene: In the present study promoter (185 bp) and coding regions (1029 bp) of the *MC1R* were sequenced from different camel breeds using different sets of primers. Mc end B up and Mc end C low primer set revealed

single nucleotide polymorphism (C to T) at position 901 after start codon. This Pro/Leu variation was detected in Kohi breed, where as rest of all the five breeds were without this polymorphism.

Based on the analysis of the Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP), Kohi breed showed the frequencies of TT, TC and CC genotype animals 0.64, 0.14 and 0.22 respectively, where as, all other breeds showed only "CC" genotype animals. All three genotyped animals showed differences in their coat colour appearance. It could only be postulate that C901T mutation could play a role in the coat colour in Kohi camels, further investigations would be needed. Dromedary MC1R had a high homology 89% with pig (Cho *et al.,* 2003) than other farm animals e.g. sheep 88% (Vage *et al.,* 1999), cattle 86% (Rouzaud *et al.,* 2001), goat 87% (Klungland *et al.,* 1997) and horse 86% (Rieder *et al.,* 2001).

Tyrosinase (TYR) gene: In this study an 820 bp fragment was sequenced in the exon 1 of the camel tyrosinase gene. The resulting camel tyrosinase sequence showed a high homology 84.2, 88.7, 89.3, 90.1, and 91.9% to the corresponding sequences of mouse (Lavado *et al*., 2005), human (Gotoh *et al*., 2004), horse (Wagner and Reissmann, 2000), cattle (Schmutz *et al*., 2004) and pig (Siebel *et al*., 2000), respectively. Sequencing animals from different breeds a single nucleotide polymorphism "C → T" was detected with primers pair C1 up and C1 low at position 113 bp after ATG. The variation provoked an amino acid variation (Pro/Leu) and created *Btg* I restriction site. By sequencing camels of different breeds a single nucleotide polymorphism (C/T) on position 200 after ATG causing an amino acid substitution (Pro/Leu) was detected. A restriction site for *Dde* I provoked by the "C" variant of this mutation that was used in a special restriction fragment length polymorphism analysis (PCR-RFLP) for genotyping of 157 animals from all six breeds of camels. Statistically significant differences in the genotype frequency between the breeds were estimated. The Sakrai breed showed higher frequency ($P < 0.05$) of homozygote without restriction (TT = 0.40) than the Marecha, Dhatti, Larri and Kohi breeds.

Growth Hormone Gene: In the present study a 539 bp fragment of growth hormone gene has been sequenced using one primer pair. Sequence analysis revealed a single nucleotide polymorphism (SNP) with a C to T variation, where nucleotide "C" furnished cutting site for *Msp* I restriction enzyme. Restriction reaction resulted in three

types of genotypes, viz. homozygous without restriction (TT), heterozygous animals (T/C) and homozygous with restriction (CC). Camels from all six breeds were screened and statistically analyzed. No significant difference was observed among the genotype frequency of various breeds.Multiple sequence analysis of dromedary growth hormone gene fragment and that of other farm animals has revealed a high homology 100% with camel (Maniou, 2003), 84 % cattle (Gordon *et al*., 1983) and 86 % horse (Zhang *et al*., 2004) growth hormone sequences.

Myogenic Factor 5 (MYF5) Gene: In the present study a part of exon 1 (422bp) of the dromedary myf-5 gene has been sequenced using the primer pair MYF 512 up and MYF 511 low. Sequence analysis revealed a single nucleotide polymorphism (SNP) in the coding sequence of MYF-5 gene at 111 bp after ATG. The frequency of genotypes among 12 animal was N = 0.42, C = 0.33 and T = 0.25. Multiple sequence alignment of dromedary MYF-5 and that of human, cattle and dog has showed 91 %, 94 % and 86 % homology respectively. Different clusters computed at phylogram could be due to the different uses (or three more different evolutions) of the species.

Myostatin Gene: In the camel myostatin gene was amplified using MN-1B up and MN-1B low primers. It was only a part of the exon 1 comprising of 256 bp. No polymorphism was observed among various camel breeds at this part of the gene. However, more than 90% homology of dromedary myostatin was observed with that of the cattle 94% (Kim *et al*., 2002), sheep 94% (McPherron and Lee, 1997) and pig 98 % (Voelker and Wheeler, 1999) sequences published in the GenBank. Big cluster in the phylogenetic tree revealed the camels as a separate species like wise pigs but near to sheep and cattle, which showed a small cluster indicating these two breeds, were having a small number of mutations in this gene since their evolution.

It is conceivable that sequence analysis of genes for coat colour phenotype, muscle development and general growth provided although some basis for differentiation. For instance, presence of MC1R allele observed in Kohi camels only and presence of various SNPs. Frequency distributions of the SNPs was found to be quite normal in camels as do for other species, but they could not be used as a breakthrough in identification of a particular breed.

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Reproductive efficiency of a non-traditional milk producing animal: The dromedary camel

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Introduction

Dromedaries are believed to have reduced fertility compared to other domestic species. Intensive reproductive management using modern methods is only applied in embryo transfer programs for racing camels. Recently, a large-scale camel milking farm was opened in the U.A.E. to produce and market fresh camel milk and added value products. The success of the operation depends on many factors, but one of the most important is reproductive efficiency. The aim of this abstract is to summarize the results of the reproductive management program of the farm in the first breeding season (2006-2007).

Materials and Methods

During the 2006-2007 breeding season, 205 dromedaries were included in the breeding program using 8 males. The management program included regular rectal examinations with ultrasonography (Aloka 500, 5 MHz, Japan) to monitor follicular development and ovulation, determine optimal time of mating, regular pregnancy diagnosis and follow-up of pregnancies with progesterone determination (Ridgeway Science, UK). No synchronization methods or induction of ovulation were applied. All parturitions were attended and calves were closely monitored. All reproductive data and history (age, breed, lactation status, body condition) were recorded and used for analysis.

Results and Discussion

1. Per-cycle and end-of-season pregnancy rate of lactating dromedaries

From September until May (8 months), the 205 dromedaries were mated in 454 follicular wave cycles ("cycle"). Breeding resulted in 196 (43.2%), 214 (47.1 %) and 44 (9.7%) pregnant, non-pregnant and non-ovulated cycles, respectively. The overall per-cycle pregnancy rate was 43.2 %. Of the 205 camels, 177 (86.3 %) were pregnant at the end of the season. This required a mean of 2.56 matings per camel. Ninety percent (183) of the camels were mated 1 to 4 times and 90 % (166) became pregnant. Ten percent of

the animals (22) were mated in 5 to 10 consecutive cycles, and 50 % of those camels conceived (11). Our overall per-cycle pregnancy rate is similar to what has been reported in New World Camelids (Vaughan and Tibary, 2006).

2. Factors affecting pregnancy rate

2.1. Month of mating (season): had an important effect on per-cycle pregnancy rate but it was not significant (P>0.05). The highest per-cycle pregnancy rates (>45 %) were achieved from November until February, with a peak of 58.1 % in December (**Fig 1**). The monthly difference could be the results of several factors such as seasonal change in male fertility and/or in ovulation rate.

2.2. Effect of male: per-cycle pregnancy rate of most males ranged between 40 to 52 %. Some males had lower fertility. Per-cycle pregnancy rate of the most and least fertile bulls were 52.8 % (38 of 72 matings) and 28.8 % (15 of 52 matings, P<0.01), respectively. The mean (±SEM) sitting time was 351 (±10.3) sec. Mean sitting time was significantly longer from December to March compared to the beginning and end of the season (P<0.001), but it did not influence per-cycle pregnancy rate.

Figure 1: Effect of month of mating on per-cycle pregnancy rate

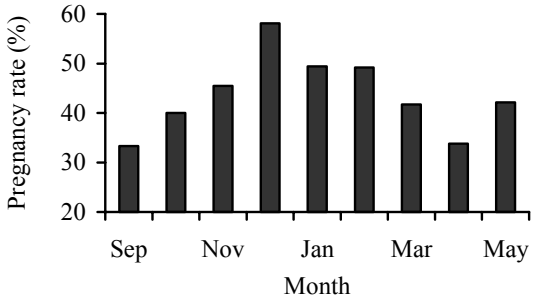
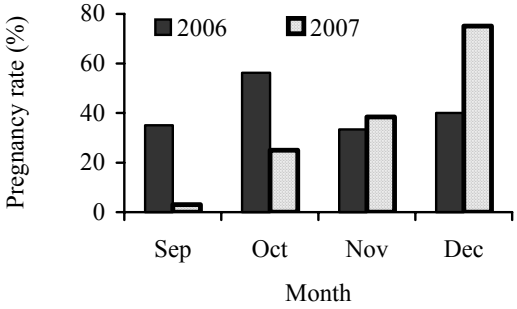


Figure 2: Per-cycle pregnancy rate of a male in 2 consecutive seasons (**P<0.01**)



2.3. Age and body condition did not influence per-cycle pregnancy rate in this group of camels. The mean (±SEM) age for pregnant and non-pregnant camels was 10.0 (±0.18) and 10.3 (±0.21) years, respectively. Per-cycle pregnancy rate was higher in *lactating* (49.8%, 115 of 231) compared to *dried* camels (40.8 %, 82 of 201, P<0.05). Per-cycle pregnancy rate of local, *racing breed* was 41.8 % (104 of 249) compared to 57.7 % (86 of 149) in *milking breed* (P<0.01).

2.3 Environment, stress/adaptation: Two males had significantly different per-cycle pregnancy rate in 2 consecutive breeding seasons ($P<0.01$). At the beginning of the 2nd season (2007), these males were kept in open paddock without shade (send $T \geq 60$ °C). Their fertility was seriously impaired but was returned to normal after changing their location and gradual decrease in ambient temperature (**Fig 2**). It seems, similarly to other species, testicular function of male dromedaries can be adversely affected by high temperature. Female dromedaries are stressed when introduced to the new management/breeding system (soft feces). Mating these camels for the first time usually does not result in pregnancy. Following adaptation, these camels have similar fertility then other females.

3. Multiple follicular wave development, double ovulation and twin pregnancies

In 32.9 % (148 of 449) of the follicular waves multiple dominant (98 % double) follicular development was recorded and 75.7 % (112) of these waves had double ovulation (24.9 % of all waves). In 42 waves (9.4 %) ovulation did not occur after mating. There was a marked effect of season on the incidence of double ovulation and on the failure of ovulation (**Fig 3**). Twin pregnancies were recorded in 8 dromedaries (7.1 % of double ovulations), but only 3 delivered (37.5 %, 1 twin). Other twin pregnant camels had EED (2), twin (2) and single (1) abortion. It seems that in dromedaries, like in mares (Ginther 1992), there is an efficient twin reduction mechanism.

Figure 3. Failure of ovulation and double ovulation in different months

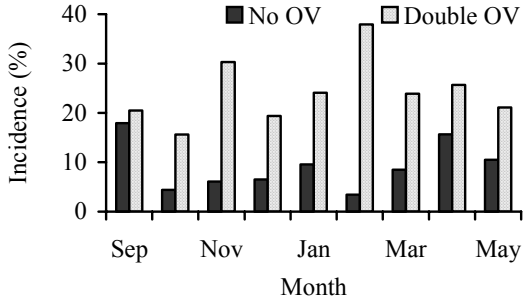
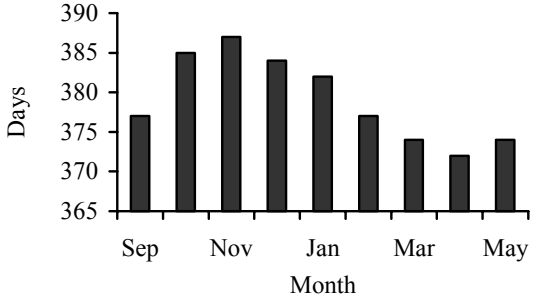


Figure 4: Effect of month of mating on length of gestation (**P<0.001**)



4. Reproductive loss during pregnancy and parturition, life-birth rate

The incidence of *Early Embryonic Death (EED)* was 10.2 % (20 of 197 pregnancies). It occurred at an average of 56.3 ± 4.06 days (SEM, 33-97 days). Twelve of 18

dromedaries (66.6 %) with EED conceived in the same breeding season. There were 4 *abortions* between 281 to 295 days and 5 *stillbirth* between 322 to 367 days. Three calves were lost at parturition due to posterioir presentation. The overall fetal loss rate was 6.8 % (12 of 177). The life-birth rate was 80.5 % (165 of 205). This result seems to be at the top of the range reported for dromedaries. In general, life-birth rate in traditional systems is much lower (< 40 %, Tibary et al, 2005).

5. Characteristics of gestation and normal parturition in dromedaries

The average length of gestation was 378.9 ± 0.7 days (SEM, 348-403 days). The month of mating (photoperiod) significantly influenced the length of gestation ($P<0.001$). The length increased with short days and decreased again with long days (**Fig 4**). The ratio of male and female calves was 57.6 % and 42.4 %, respectively. The average birth weight was 37.6 ± 0.4 kg (SEM, 16.4-51 kg), and there was no difference between male and female calves. Most deliveries (60 %, 100 of 168) took place in the afternoon during daylight, 20 % and 20 % occurred during the night and in the morning hours. Distochia that required cesarean section was only caused by posterior presentation (1.8 %, 3 cases).

Conclusion

We have shown that in practice dromedaries can be as fertile as any other domestic species if appropriate reproductive management program is applied. We have also been collecting valuable new data on various aspects of reproductive physiology. This work has tremendous financial benefits for the organization as it gives us the tool to synchronize production with market demands and life-birth rate is nearly doubled compared to traditional systems.

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Reproductive efficiency and disorders of the reproductive tract in alpacas

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Reproductive efficiency

Alpacas are as fertile as other domestic livestock. Conception and birth rates in alpacas average more than 50 % from a single mating when mating system, fertility and body weight of sire and dam, post-partum interval, environmental conditions and nutrition are taken into consideration (Vaughan and Tibary 2006). This translates into 90 % of females conceiving in the first three mating attempts after reaching puberty or post-partum. Sexual receptivity, ovulation, fertilisation and embryonic survival rates in alpacas are similar throughout the year (Fernandez-Baca, Novoa et al. 1972; Knight, Death et al. 1992; Vaughan, Macmillan et al. 2003).

The ideal mating time appears to be during the late growing and early mature phases of follicular growth when follicles are most likely to ovulate, approximately 8 days after new wave emergence (Vaughan, Macmillan et al. 2004). Unfortunately, there is no association between sexual receptivity and follicular status or ability to conceive (Vaughan, Macmillan et al. 2003) and therefore farmers have no simple measure of when a female may be more likely to conceive. Paddock mating may allow the male to determine an optimal time for mating, but when males and females are together in the same paddock continuously, male libido may decline within a week (Sumar 1999). Where supervised yard-matings occur, the first two matings by a male on any day are equally as likely to result in pregnancy (Vaughan, Macmillan et al. 2003). While more matings per day by a male are possible, conception rates decline and depend on the fertility of individual males (Bravo, Flores et al. 1997).

Male fertility plays a key role in reproductive efficiency in alpacas. Sperm production is correlated with testicular weight and testicular length in alpacas and Table 1 indicates how mean testicular length may be used to estimate the likelihood of sperm production (Galloway 2000). Selection of early maturing males with large testicles should ensure optimal conception rates in any alpaca herd. Both genetics and environmental influences govern age at puberty and testicular size.

Table 1. Development of testicular function in alpacas with testicles of different length (Galloway 2000).

Mean testicular length (cm)	Proportion of males (%)	Percentage of testicular tissue producing elongated spermatids
<3	100	0
3-4	68	<10
	31	30-60
>4	36	<10
	31	10-60
	31	>60

Embryonic death is estimated to affect 10-15 % of all alpaca pregnancies in the first 60 days of gestation, while foetal loss after 60 days has been estimated at 5 % of all pregnancies (Vaughan and Tibary 2006). Causes include defects in maternal recognition, implantation failure, hormonal imbalances, nutritional deficiencies, handling/transport stress, systemic disease and chromosomal abnormalities. Infectious causes of abortion in camelids generally include toxoplasmosis, brucellosis, chlamydiosis, listeriosis, leptospirosis and neosporosis (Vaughan and Tibary 2006).

Disorders of the female reproductive tract

Female alpacas are usually presented for infertility examination because they are unable to conceive following multiple matings, they have aborted, they have visible abnormalities of the external genitalia or they are continuously rejecting the male (Vaughan and Tibary 2006).

Table 2. Disorders of the female reproductive tract in alpacas

Ovary/bursa/oviduct	**Uterus**	**Cervix/vagina/vulva**
ovarian hypoplasia*	segmental aplasia*	double cervix*
paraovarian cysts*	uterus unicornis*	segmental aplasia*
hydrosalpinx*	uterine hypoplasia*	persistent hymen*
teratoma*	mucometra*	vulvar aplasia*
ovulation failure	endometritis	infantile vulva*
persistent corpus luteum	endometrial fibrosis	intersex*
anovulatory large follicles	pyometra	adhesions post-dystocia
oophoritis	peri-uterine adhesions	cervicitis
granulosa cell tumour	uterine cysts/abscesses	
salpingitis	uterine adenocarcinoma	
pyosalpinx		

*congenital condition and should not be used for breeding

Diagnosing the cause of infertility requires a full reproductive history, a general physical examination and thorough evaluation of the reproductive tract using transrectal ultrasonography, vaginal examination, uterine cytology/culture/biopsy, laparoscopy and chromosomal testing. The congenital and acquired conditions of infertility described in the female alpaca are listed in Table 2.

The most common cause of infertility in a study of approximately 150 infertile females was ovarian hypoplasia (Sumar 1983). The condition may be partial or complete, unilateral or bilateral. Animals with complete bilateral hypoplasia will have no primordial follicles and are therefore sterile, but females with partial hypoplasia should not be used for breeding as the condition is likely to be heritable. Segmental aplasia of the uterus, cervix or vagina is usually accompanied by mucometra as there is no outlet for glandular secretions produced by uterine glands.

Common causes of acquired infertility include endometritis, persistent corpora lutea and dystocia-related trauma resulting in secondary fibrosis of the uterus, cervix and/or vagina. Thorough examination of the reproductive tract will allow diagnosis and treatment of these conditions.

Disorders of the male reproductive tract

It is essential to establish whether a male is sexually mature during an infertility investigation as age at onset of puberty in alpacas ranges from less than 12 months of age to greater than 4 years of age. Breeding soundness examination of infertile male alpacas includes collection of a thorough breeding history, a general physical examination and palpation and ultrasonography of the scrotum, testes and prepuce (Tibary and Vaughan 2006). Testicular length is a very important indicator of sperm production ability and fertility (Table 1). Testicular biopsy, mating ability and semen evaluation will complement the examination.

Testicular hypoplasia was the most common cause of congenital abnormality in the male reproductive tract in a study of more than 3000 alpaca males (Sumar 1983). The condition is usually bilateral, but may be unilateral, total or partial. Histologically, there is a reduced number/absence of seminiferous tubules and no spermiogenesis. The condition is suspected to be inherited in alpacas and affected males should not be used for breeding.

Testicular degeneration is probably the most common cause of acquired infertility and results from heat stress, trauma or chronic inflammation of the testes secondary to

severe systemic disease (Tibary and Vaughan 2006). The testes are usually smaller than normal and may be soft for very firm and fibrous. The testes may recover following degeneration if the cause is removed but long-standing degeneration may further deteriorate into testicular atrophy.

Table 3. Disorders of the male reproductive tract in alpacas

Scrotum/Testis	Penis/prepuce	Epididymis
testicular hypoplasia*	urolithiasis	epididymal cysts
cryptorchidism (intra-abdominal)*	balanoposthitis	
ectopic testes (extra-abdominal and extra-scrotal)*	paraphimosis	
monorchidism*	preputial prolapse	
testicular cysts*	preputial swelling	
testicular degeneration, orchitis		
Hydrocoele, seminoma, scrotal trauma		

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Effect of management system on Sudanese camels calving interval

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Introduction

The camel is a very important animal in the dry regions because of its ability to provide milk, meat and transport for people under these climatic conditions. In Sudan, camels are traditionally reared in extensive area with low feed quality and availability. The reproductive efficiency of Sudanese camels under Pastoral management (traditional) is low. The Calving interval is varying between 28 to 36 months. Low reproductive performance in camels is mainly due to a delayed puberty, long Calving interval, limited breeding season, herd dynamics and lack of sufficient feed.

The aim of the present study was to investigate the impact of improved management system on camel calving interval.

Material and Methods

Eighteen (18) she-camels in late pregnancy and two mature males for mating were used to determine the effect of Management System on calving interval, in North Kordofan State (Western Sudan). The camels were selected randomly from Nomadic herd and maintained under two management systems after calving. Group one (N = 9) reared under semi-intensive management: herded during night in closed pens, in addition of natural pasture they allowanced supplementary diet (2 kg concentrates + 5 kg roughage /head/day), watering *ad-lib*, health care, internal and external parasites control were applied. Group two (N = 9) reared under Traditional system, depending on natural rangeland and unsupplemented with exception of salt, water regime (6-7 days) was practiced.

In both systems the calves were fellow their dams and suckling was available for the half of the udder during the day. Weaning was depending on pregnancy advance and normally was done by traditional methods. The experimental females in each group kept

together with the bull during 18 months the mating were applied naturally without any assistance. Blood samples (N = 252) were collected from jugular vein since 4-months post partum and continued 14 successive months at monthly interval. The serum samples were separated and stored at -20°C until hormone assay were performed, progesterone concentration was determined by specific radio immuno assay (RIA) kits. (Diagnostic Products Corporation, INRA laboratory, France). The progesterone level was compared with behaviour signs of she camel (erect and curving her tail when owner or male coming near her, refusing the male, raising head). The calving interval was calculated by adding the gestation period (12 month) to the period from calving till she camel became pregnant.

Results

Under semi-intensive management during post-partum and early lactation period camel's reproductive traits were improved.

The ratios of pregnant vs non-pregnant during total experimental period (18 months) in semi-intensive and traditional management were 8:1 and 4:5 respectively.

The calving interval was shortened under semi-intensive system. In group 1 seven females became pregnant in the period between 5 and 8 month post-partum and the calving interval varying between 17 to 20 months. An additional one became pregnant on 13th month and calving interval was 25 month. In group 2 (traditional system) three she camels became pregnant during the 11 and 16 month post-partum and the calving interval varying between 23 to 26 months, one she camel became pregnant after 17 month post-partum and the calving interval was 29 month.

In pregnant females Progesterone concentration increased significantly ($P<0.05$) during early months of pregnancy to a value above 2 ng/ml blood. During pregnancy the value is increased to an average value of 5.8 ±1.45 ng/ml blood over a period of 8 months followed by a strong decrease during the last two months before calving (see figure 1 and 2).

Conclusions

The findings of the present study assume that the low rate of fertility in the camel under traditional system in Sudan is considered mainly as being due to the general lack of fodder and the poor nutritive value of the natural pastures and water scarcity. This

might indicate that in equatorial regions forage and water availability is the major factor governing seasonality of mating and births in camels.

Additional feeding of 2 kg concentrates and 5 kg of roughages per day during the lactating period shortened dramatically the calving interval and increased rate of pregnancy. We calculated that it would be possible to expect above 3 times more young calves per year. Blood progesterone can be a valuable tool for assessing early pregnancy in camels coupled with the sensory observations.

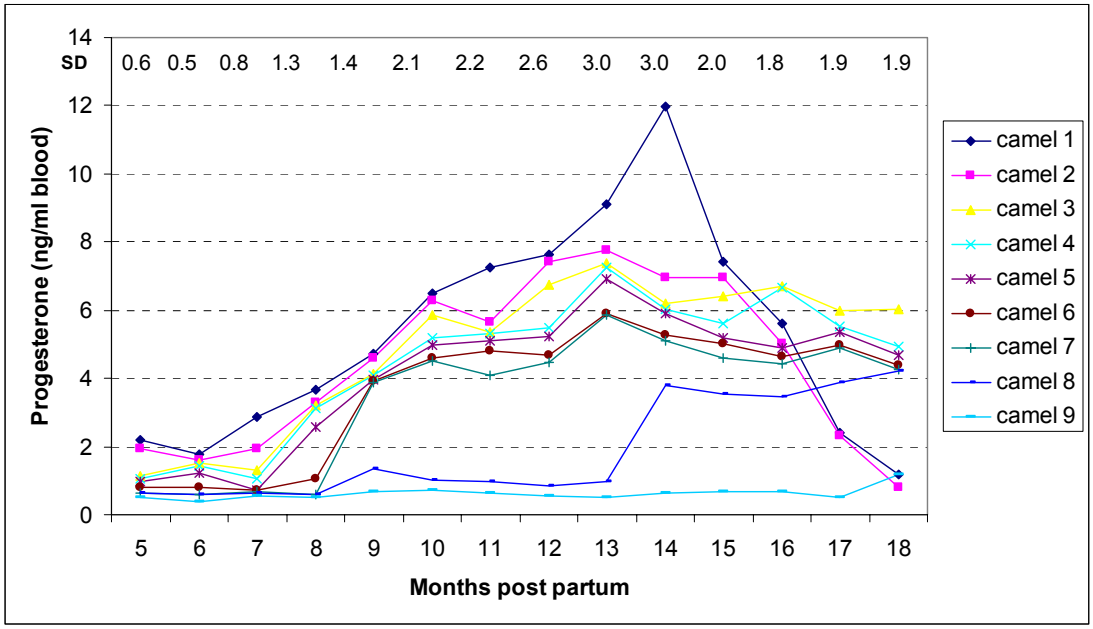

Figure 1: Progesterone concentration (ng/ml blood) on camel under semi-intensive management during the experimental period

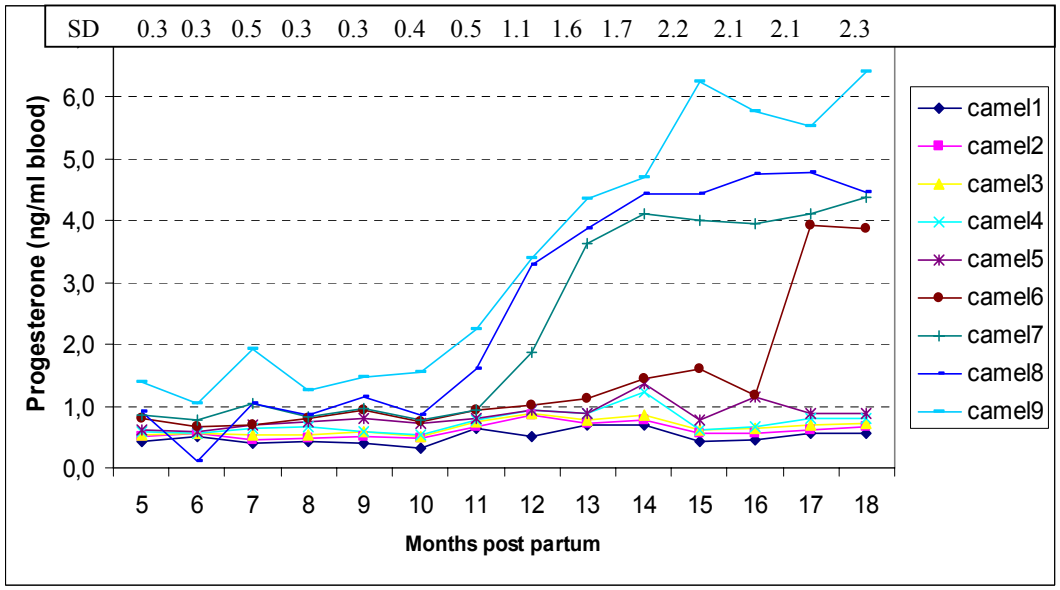

Figure 2: Progesterone concentration (ng/ml blood) on camel under traditional management during the experimental period

Reproductive performance of one-humped camel (*Camelus dromedarius)* at the Komodugu-Yobe River Basin, Nigeria

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Introduction

Camel production could be a profitable venture for utilizing the vast arid and semiarid areas of Northern Nigeria where other animals thrive with difficulty, especially due to the recurring drought conditions. Full exploitation of camels for milk and meat production would only be possible when their reproductive performance is properly understood and improved. The maintenance of high levels of reproduction in camel is essential not only for profitable production but also to provide ample opportunities for selection and genetic improvement. Unfortunately information on economic and biological evaluation of camels in terms of productive and reproductive potentials in Nigeria is sparse. The aim of this paper was to study reproductive parameters of camels kept under pastoral management at the Komodugu-Yobe River Basin, Nigeria.

Materials and Methods

The study was conducted at 8 different locations at Geidam local government area along the Komodugu-Yobe river basin, Nigeria (11^0 30 to 12^0 00E, 12^0 30 to 13^0 00N) in 2007.

Visits were made to sixteen pastoral herds that were selected on the basis of the nomads' willingness to participate and provide information. Physiological status (dry, lactating or pregnant), previous breeding history were obtained by questioning the herd using the progeny history questionnaire (Kaufmann, 2005). The deduction of annual calving rate from a day examination of herd requires two sources of information: (1) number and estimated ages of calves; (2) the number of camels found pregnant. Aging of the camels were done by combination of using the eruption of the permanent incisors and questioning of the herd owners as to the age of their animals. It was observed that the result of the two methods were generally in close agreement. This highlighted how well the owners knew their animals individually.

The camels were managed traditionally where bulls and cows were allowed to roam freely. Camels were milked twice a day, in the morning before grazing and in the evening. Calves were weaned naturally by their dams.

Definition of parameters used:

Crude death rate % = No. of deaths / average herd size x100

Young Stock death rate = No. of deaths of animals < 1 year / No. of live births x 100

General fertility rate (%) = No. of live births / No. of females of reproductive age (4years & older) x 100

Other aspects of reproduction taken into consideration were seasonality of breeding (conception and calving). A total of 1460 camels in 16 herds were examined. Herd size therefore average 91.25.

Results and Discussion

The mean age at first calving and calving interval were 5.17±0.94 and 2.82±0.77 years respectively. The effect of location on these parameters were not significant as shown in Fig 2. The results are in conformity with the reports of Kaufmann (2005) in Kenyan camels.

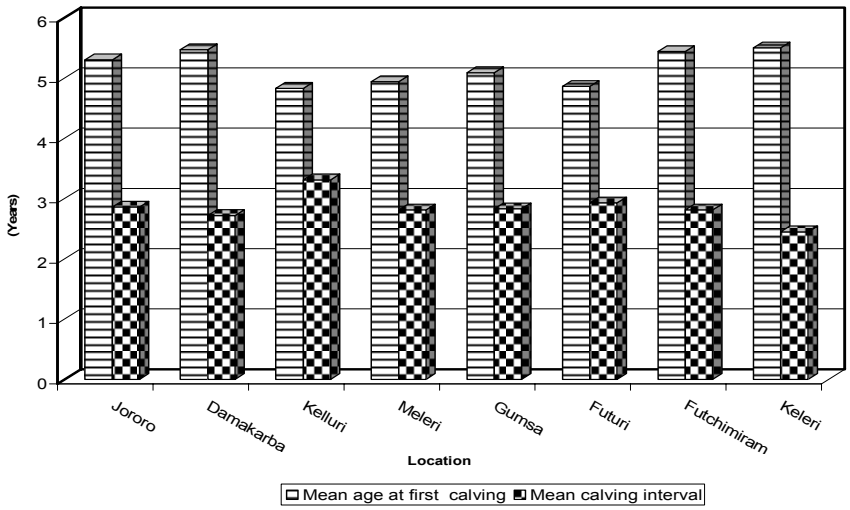

Fig 2: Mean age at first calving and calving interval of traditionally managed camels

The general fertility was 41.78%. These parameters were similar across the study herds and sites. The abortion rate ranges between 0 to 50% with a mean of 26.71%. The very high abortion rate for Jororo camels is not very clear, the nomads seem to indicate that pregnant camels in the first trimester that feed on some plants such as the pods of

Leptidonia lacifolia that are believed to cause abortion. The *Leptidonia lacifolia* vine is abundant in the area and highly relish by camels. Abortion due to brucellosis should not be entirely ruled out since there were reports of Brucellosis in camels in Nigeria previously (Kudi et al., 1997). The calves' crude death rate ranges between 0 to 8% with a mean of 3.32%.

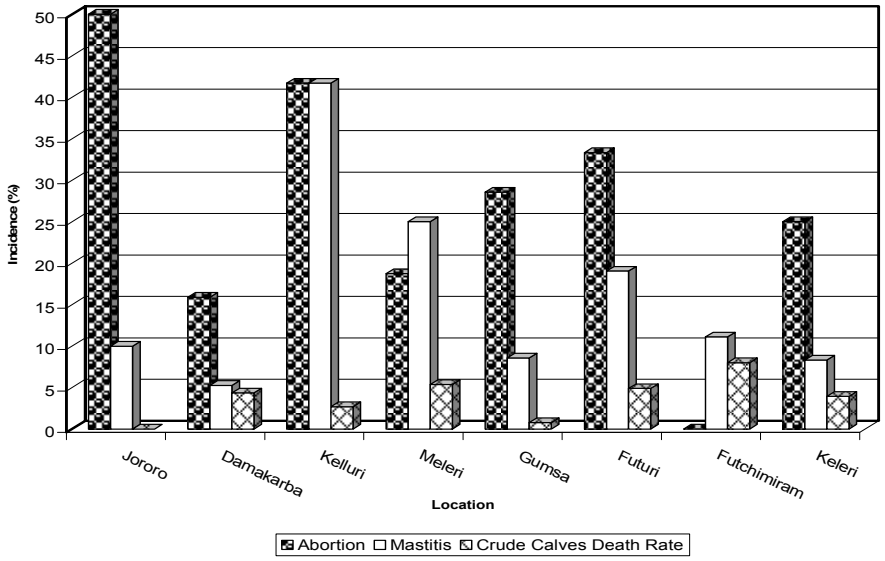

Fig 3: Incidence of abortion, mastitis and crude calve's death rate of traditionally managed camels

The crude rate is within expected range for pastoral herds (Baumann and Zessin 1992). The main causes of calf mortality are calf diarrhoea in the rainy season and mange. The milking ability of camels was influenced by location ($P<0.05$) with camels from Futuri having higher milking ability. The mean daily milk yield per dam was 2.55 litres. The incidence of lactating camels having mastitis was 14.4% this high value might be associated with the practice of tying 2 teats of the udder with soft sparks to prevent calves from suckling as shown below.

The status of the camels studied is shown in Fig 4., 52.78, 41.78 and 5.48 % pregnant, lactating and dry camels respectively. The trend was statistically similar in the locations studied. It is often desirable to advice farmers, especially peasants who rely solely on natural mating on the right time and condition to breed their camels for chances of conception. Hence there is a need for a good knowledge of the breeding season. Based on calving distribution (Fig 5.) there

was a significant seasonal variation in seasons of calving with 83.7, 12.4 and 3.9% of calvings based on 345 calving records, during cold dry season (November – February) hot dry season (March - July) and rainy season (June – October). It thus fallows that given the gestation length of about 13 months that camels had conceived within the season to which they had calved the previous year. Sghiri and Driancourt (1999) reported similar seasonal breeding and calvings at Morrocco. There has been no previous report of camel breeding seasons in Nigeria, more studies however need to be conducted to establish the causes of seasonality.

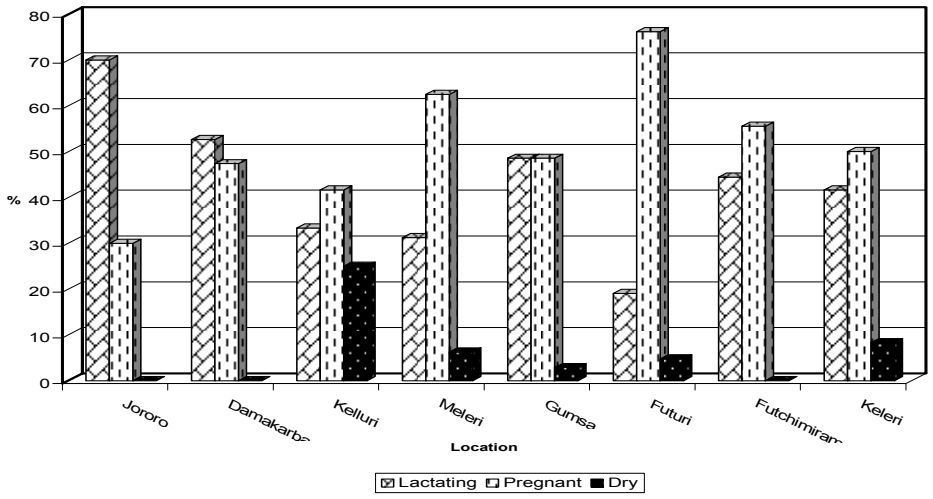

Fig 4: Physiological status of the camels studied

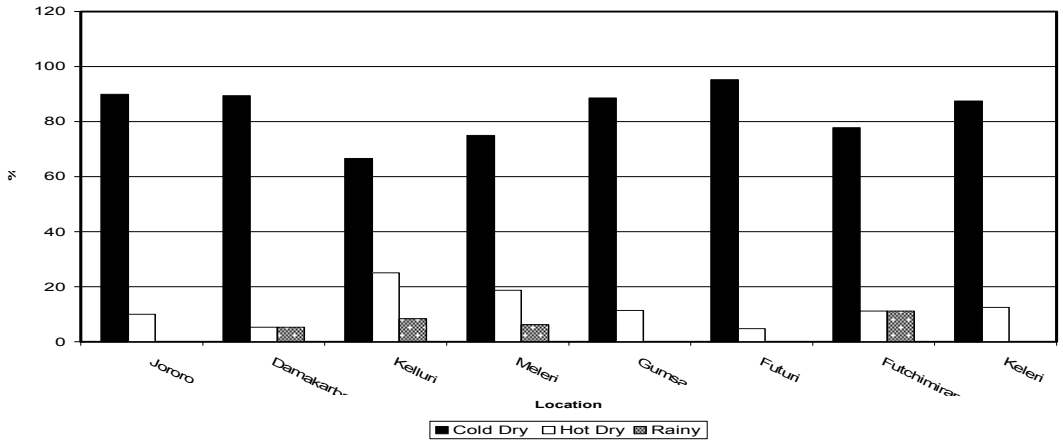

Fig 5: Seasonal variation in calving

Conclusion

The maintenance of high levels of reproduction in camel is essential not only for profitable production but also to provide ample opportunities for selection and genetic improvement. Efforts should be geared towards limiting abortion and pre weaning mortalities rates among these traditionally managed camels. Detailed studies should be conducted to minimize causes of reduced reproductive rate at herd and individual levels.

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The use of laparoscopy in tandem with ultrasonography for diagnosis of internal gynaecological defects in camels

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The Gynaecology Clinic of the Veterinary Teaching Hospital at King Faisal University- Saudi Arabia receives annually about 2000 female camels (about 9 camels per working day) suffering from various gynaecological problems. Owners travel hundreds to thousands kilometres from the spacious desert lands of Saudi Arabia and other GCC countries to the hospital seeking distinguished diagnosis and treatment of their camel illness. The objectives of using the rigid endoscope in combination with ultrasound array scanner in the Gynaecology Clinic were to improve the success rates of gynaecological diagnosis and treatment. The genital tract of the non-pregnant female camel is directed horizontally (similar to that of the cow). The left uterine horn is always larger than the right horn. The ovaries are located just lateral to the left and right horns. The ovaries of the non-pregnant camel contain variable numbers and sizes of follicles. The corpus luteum is usually palpated at pregnancy. However, some female camels show corpora lutea in a non-pregnancy state following embryonic death and pyometra. Manual rectal examination in female camels tentatively assessed the anatomical and physiological activity of the ovaries, Fallopian tubes, uterus and cervix. The uterus was felt rigid during oestrus, but soft during the non-follicular phase of the oestrous cycle. The uterine tubes were not usually felt unless they were thickened due to inflammation or occlusion. Rectal examination was performed with the animal restrained on sternal recumbancy. Each carpal joint was fixed by a rope and both hind fetlocks were fixed through passing a-two meter rope at the fetlocks along the lumbar vertebrae. However, the use of the real time ultrasound array scanner improved the diagnostic procedure to measure the ovarian structures, confirm pregnancy and detect some pathological conditions. The scanner used was a notebook B mode with a 7.5 MHz and digital camera. The rectum was emptied of faecal material to ensure a good contact between the transducer and the rectal wall. The transrectal probe was lubricated and passed with the hand through the rectum over the genital organs in a longitudinal

manner. The use of the scanner alone did not achieve a complete picture of the internal genital organs. The use of the laparoscope allowed visualization of the internal genital organs in female camels. Rectal examination alone or with the scanner did not need tranquillity of the female camel. Laparoscopy has given more opportunities to trace gynaecological defects which neither rectal palpation nor scanning succeeded in monitoring. Disregarding the cost of the scanner and endoscope, no special preparation of the animal was needed prior to scanning, however, laparoscopy was preceded by rigorous preparations, such as shaving, surgical asepsis, anaesthesia, cannulation, carbon dioxide insufflation, and assistants. The optic system consisted of a rigid endoscope, a fibre optic cable, a video camera, a light source and a TV monitor. The insufflation system permitted the creation of a pneumoperitoneum by insufflation gas in the abdomen at rate of 15 L/min. The insufflator was connected to a CO_2 tank. Laparoscopy was performed on camels positioned in sternal recumbency. Following sedation, the left flank paralumbar fossa was approached. The surgical field was prepared using routine aseptic technique. Following a small cut by a plate, a secured trocar/cannula system was used to penetrate the abdominal wall and allowed the introduction of the endoscope. The trocar was a pyramid cutting edge with a spring-loaded safety shield. When the trocar was pushed, care was taken to avoid spleen and other internal organs injury. The abdominal and pelvic cavities were inflated with carbon dioxide to achieve adequate spacing. Following removal of the trocar, the cannula was placed through the abdominal wall and the trocar was replaced with a 90 degree endoscope. CO_2 was insufflated at 15 mm Hg. The use of the laparoscope in tandem with the scanner helped in strengthening the diagnosis of various gynaecological abnormalities, such as congenital defects, ovarian cysts, ovarian adhesions, tubal swellings, tubal adhesions, infundibular cysts, and uterine adhesions. The uterine tube defects were not uncommon. A high number of female camels suffering from infertility have been diagnosed to have variable number and sizes of infundibular cysts. They were either unilateral or bilateral. The owners of such conditions have been advised to cull their female camels, since these cysts blocked both tubes, and hence the animal has been considered sterile. Surgical interference to remove the cyst was not recommended, since it has been shown in a previous study that the cysts harbour zoonotic bacteria. i.e. *Aeromonas hydrophilla.* Our plan in the near future is to extend our services to laparoscopic surgery, which has been considered a key-hole surgery.

Challenges in the development of a large-scale milking system for dromedary camels

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Introduction

Dromedaries are an important source of milk in rural areas of many arid countries (Faye 2005). However, an extensive production system can not guarantee constant quantity and quality of raw camel milk for urban markets. The idea of an integrated camel milk production, processing and marketing system was born in Dubai few years ago and was followed by the establishment of the world first large-scale camel milking farm. The aim of the project is to develop and maintain biologically, environmentally and financially sustainable, intensive camel milk production and market high quality milk and milk products. We have been facing many challenges over the years while setting up the farm that are summarized in this abstract.

1. Availability of camels, production potential, genetic backgorund

Intensive production requires concentration of animals. This can be achieved by purchasing camels individually or in lots from small farmers. This is a crucial and laborious process that determines the initial success of the operation. However, adult camels preferably with calf at foot, available for sale, are difficult to find in large numbers. One might need to look for camels in different countries that raise questions concerning international trade and animal health issues. Even if camels are available, most of the time there is no information on production potential. In general, there is an important variability in individual milk production within the same breed and females of so-called racing breeds could produce more milk than camels of so-called milk breeds.

2. General and udder health status of the animals, infectious disease control

In practice, most of the time proper pre-purchase examination of milking camels can not be performed. For this reason, it is important to establish and maintain a quarantine area/farm where camels from different locations are brought together and health status of the animals is thoroughly evaluated. Priority is given to infectious diseases status (such as Brucellosis, Tuberculosis, Surra, FMD etc.). Camels confirmed

positive for OIE list diseases are separated and removed from the quarantine and handled according to local regulations. Many dromedaries could be rejected as in some geographical areas i.e. the prevalence of Brucellosis exceeds 30 % of the camel population. Some diagnostic tests are not validated for Camelids and could result in false positive reactions (Cousins and Florisson, 2005). Udder health should also be monitored carefully. In previously hand milked multiparous camels, teats are frequently enlarged and deformed or udders are infected with pathogenic bacteria (Staph. aureus, Str. agalactiae, Str. bovis etc.). In a recent survey, 31.6 % (19 animals) of 60 new lactating camels were infected. Those camels should be either rejected or treated before starting on machine milking.

3. Development of milking technology (milking stands, equipment, milking liners)

Large-scale camel dairy farming requires automatic milking system. First, milking stands, milking parlours had to be designed that are comfortable and suitable for different sizes of camels. Our camels are milked in a 2x24 herringbone parlour. Standard milking equipment used for other species is commercially available and could be used for dromedaries. However milking liners and claw must be carefully selected or developed for camels. There is a huge variation in size of teats between camels (length = 7.1 ± 2.22 cm, mean±SD; max: 16.0 cm, min: 2.93 cm) and only 2 cm of the teat end fits into a commercial bovine liner. In addition, the teat undergoes significant size and volume changes during milking. Length and volume increase by 50 and 170 %, respectively. For this reason, it is difficult to find appropriate, ready made liner that could be used long term for camels. In our experience, some liners are less tolerated, induce udder oedema and enhances colonisation of *Staph. aureus* compared to other liners.

4. Co-operation of animals, training camels for milking

It is a common misbelieve that camels are aggressive, dangerous and difficult to handle. Like any other domesticated species, camels can be trained for different procedures including entering the parlour and milking with positive reinforcement. But, successful training requires thorough understanding of behaviour of this species and experience. In the initial phase of the project, 30 % of the camels were rejected due to failure of training. Since our method is applied, several hundreds (>800) of camels were

trained and none of the animals was rejected for this reason. The training of a milking camel takes 2 to 4 weeks depending on the background of the animal.

5. Adaptation of milking to the physiology of milk let-down in dromedaries

Compared to cows, camels have limited cistern volume and milk let-down is usually induced by the suckling effect of the calf. In a large-scale system, milking would be very difficult to manage if calves were present in the parlour. For this reason, an efficient method of manual udder/teat stimulation should be developed to induce milk let-down. In our previous study, we have shown that stimulation time is long (mean±SD: 123.2 ± 84.4 sec.) and milking time is short (mean±SD: 126.9 ± 41.1 sec.) in dromedaries (Wernery et al, 2004). These physiological characteristics together with the morphological variability underline the importance of well trained milkers. Milking routine differs significantly from that used for cows.

6. Adaptation of husbandry and management to the needs of this species, avoid multi-factorial diseases

We have been trying to develop the large-scale production system as close to natural conditions/needs of dromedaries as possible. Dams and calves are kept in adjacent paddocks throughout lactation and are allowed together after each milking. Once a day, they go out together for a long walk. Staff is trained to take care of animals in a gentle manner to provide a low-stress environment. Camels are handled individually for different procedures (nail trimming, washing, rectal examination etc.). At this stage, it is difficult to predict what kind of multi-factorial diseases might occur in the future as a result of rapid change from extensive (free ranging) to intensive management system. All changes, especially those related to feeding should be introduced cautiously.

7. Development of a food safety system and quality criteria for camel milk

In our modern world, food safety and consumer protection is of primary importance. In the UAE, all food producing establishment must implement minimum the HACCP system. We have decided to go one step further to develop the ISO 22000:2005 Food Safety Management system that is in its final stage if implementation. The system ensures that our final product is of the highest and constant quality possible. However, first we have to define what exactly the term of “high quality camel milk”

means. Many studies are required to establish the normal range and acceptable upper limits for chemical and/or microbiological content, SCC etc. in raw camel milk and to set up standards.

8. Reproductive management to maximise breeding efficiency and reduce inter-calving interval

The primary aim is to provide constant quantity of milk throughout the year or follow market demands with production. This aim can not be achieved without precise and well planned reproductive management. It is widely accepted that fertility rate of dromedaries is low (Tibary at al, 2005). In traditionally managed local herds, the calving rate (of mated females) is below 40 %! One can easily calculate the economic losses of such a level of reproductive management for the operation. Inter-calving interval in lactating dromedaries is usually well above 24 months. Detailed studies are required to define optimal re-breeding time with maximum milk production.

Conclusion

We have demonstrated that camel milk can be produced for commercial purposes in a large-scale system, and still in an animal-friendly way. Hopefully, the success of our project underlines the importance of dromedaries in our modern society and boosts the further development of the species. However, this development can not be achieved without co-ordinated and systematic research on all fields of Camelid science.

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The impact of farming system on Sudanese camel milk production

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Introduction

Sudan ranks second country in the world on Camel population. According to last estimation of Camel's in Sudan there are 3.908 million heads (Ministry of Animal Resource and Fisheries, 2005). Milk production of camels in drought regions is a valuable source of food for the human population in these areas. In most pastoral systems, the main target of camel herding is to satisfy the family's demand of milk. In Sudan the camel herders are in a continuous move in response to availability of grazing and water supplies and camel milk is one of the main components of their basic diet. Milk yield fluctuates at a low level considerably from time of abundance to time of acute scarcity of feed and water. The aim of this study was to investigate the effect of improved management system on camel milk yield.

Material and Methods

Twenty lactating she-Camels of the Sudanese Arabi breed were selected randomly at late pregnancy from nomadic herd in North Kordofan State (Western Sudan). The experimental animals were divided into two equal groups 10 she-camels each. Group 1 was managed after calving in a semi intensive system**:** animals were herded during night in closed pens and set free during midday, in addition of natural pasture supplementation consisted of 2 kg concentrates and 5 kg roughages per head per day allowanced, water *ad libitum,* health care, external and internal parasites control was practiced. Group 2 was served as a control, was managed traditionally. On this system the animals are brought to grazing areas where they select food by themselves from the available plants and allowing nothing as supplement feeding, with the exception of offering salt, and water regime (6-7 days) was applied. The collection of milk samples (N = 480) were started at 15 days post partum and continued for 12 successive months

in biweekly intervals twice a day (approximately 12 hours interval). Hand milking was applied**.** The milker approaches the she-camels from the left side. Two teats are milked and leaving the remaining two teats for suckling by calf. The milk yield was estimated directly after milking by using measuring cylinders with an accuracy of 5 ml. The milk yield registered and total milk yields per annum were estimated. This amount represent approximately a half of the total milk yield. Over the total experimental period and according to herders practice the traditional *Sorrar* technique was used**:** To prevent the calf from suckling two teats are tied up with a soft tape of cloth removed only at milking time. Every day the position of tied up teats were change to avoid the she-camels udder harmful.

Results

The average daily milk yields were 6.85 ± 1.32 l/day and 3.14 ± 0.66 l/day for semi-intensive and traditional system, respectively (Fig.1). The results indicated that the trend of daily milk yield increased significantly ($P<0.05$) after calving until reach the peak in the 13th week post partum in both systems and then declined gradually through the lactation period. The maximum average daily milk yield was 8.7 ± 0.94 l/day and 4.30 ± 0.59 l/day in semi-intensive and traditional system, respectively. The increase of milk yield under traditional system in the week 29th is due to the availability of green fodder during rainy season in the study area. Daily milk yield range was varying between 3.7 - 10 l/day in semi-intensive system, on the other hand the range of daily milk production of the camel reared under traditional management was 1.8 -5.2 l/day.

Improving management system highly significantly ($P<0.01$) affected total milk production. The calculated milk yield per year was 2633 l/animal and 1204 l/animal on semi-intensive and traditional system, respectively. The actual milk secreted is higher than the recorded figures presented in this study, because the calves have access to the mothers during the whole day and are suckling faster than the milker is milking.

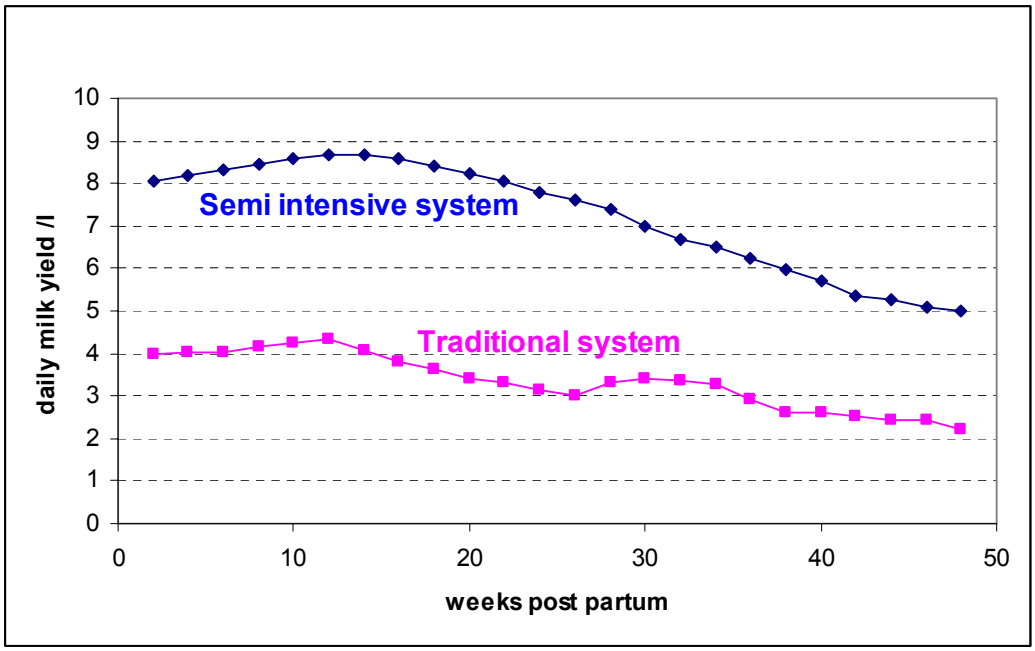

Figure 1: Average Camel milk yield under semi intensive and traditional management (l/day)

Conclusion

Camels raised under semi-intensive management were able to produce significantly more milk than the other reared under traditional system. This is attributed to the forage availability and the supplementary diets, water availability and health care that oriented to the camels in the semi intensive system. It was evident from the data that Farming system has significant impact on daily and total camel milk yield. Improving management increased milk yield 2.3 times from that producing under traditional management, which reflect good advantages to the calf and family livelihood.

Camel reproduction and production in Egypt

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1. Numbers and distribution

1.1. Numbers

Total number of camels in Egypt differs from year to year, but it increased in the last 10 years according to FAO statistics and some Egyptian Organizations as Ministry of Agriculture and Veterinary Service Organization

Table 1 . Number of camels (Heads) in Egypt from 1980 to 2006

Years	FAO	Ministry of Agriculture
2006	127000	129290
2003	120000	127500
2002	135000	127079
2001	134000	150255
2000	141000	140747

1.2. Distribution

Distribution of camels in Egypt differs from governorate to the other. Number of camels increased in desert governorates (Red Sea, Marsa Matroh, North Sinai, South Sinai and Wadi EL-Gadid governorates). The number of camels /1000 capita was 0.17, 0.57, 1.9 and 87.65 heads in the urban, lower, upper and desert governorates of Egypt, respectively. These values were calculated according to the number of population in these areas. Urban Governorates (Cairo, Alexandria, Port- Said and Suez), Desert Governorates (Red Sea, El-Wadi El-Gidid, Matrouh, North Sinai and South Sinai), (Bekele, and Zeleke (2000).

Table 2. Camel's meat production is about 6.91 % out of total red meat produced in Egypt, during 2006.

Years	Ton / l000 capital				Total red meat	Camels (Tons / 1000 capita)	Camels (% of total red meat)
	Cattle	Buffalo	Sheep	Goats			
2006	5.6	4.6	1.33	0.62	10.6	0.71	7.5
2002	3.51	4.36	1.07	0.47	9.41	0.65	6.91
2001	3.57	4.39	1.09	0.47	9.52	0.75	7.88
2000	3.77	4.24	1.08	0.48	9.57	0.58	6.06

2. Meat production

Average camels meat production / year during the year's 1987-1991 was about 0.4 % from the total meat produced in Egypt. The umber of camels slaughtered was 20000 heads. The camel meat produced was 29900, 30000 and 32890 tons during the year's 1991, 1992 and 1993. Veterinary Service Organization (2006) statistics showed that the number of slaughtered camels in Egypt during 2000 and 2006, were 109960 and 127836 heads, respectively. It is of interest to note that meat produced from camels was higher than that produced from each of goats and ducks during the last years 1999 to 2006.

3. Milk production

In Egypt, a good daily milk production of the she camel of 10-15 kg was obtained giving a yield of approximately 3000 - 4000 kg per lactation. Daily yields of 22 kg have been recorded. Daily production was only 4 kg, with a total production of 1500 kg with grazing in the deserts. Meat of young camels is comparable in taste and texture to beef. The dressing percentage of the carcass varies between 48 and 60%. The carcass % of camels is similar to carcass % of grazing cows and buffaloes. Camels younger than 5 years have less protein, fat and ash than in the older camels. Young camel's meat may be more preferable to consumers due to the decrease of fat levels in camel's meats than other animal. It may be beneficial to increase the camel meat production by increasing the number of camels.

4. Camel reproduction

Camels grow slowly, reaching puberty at a later age than other livestock species. Sexual maturity is probably reached at 3-5 years. The gestation period is about (12-13 months) with the result that the age at first calving is generally 5-7 years. Calf birth weight ranges between 30 and 36 kg. Male calves weight is slightly heavier than in females. The camel has a longer breeding life than other domestic species, and fecundity increase with age declining only with the onset senility.

The low reproductive performance is one of the most important factors affecting camel productivity. Factors contributing to low fertility in camels are many and complex, for example: the advanced age at puberty (3-4 years) and hence late age at first calving, the limited libido of males and hence limited breeding opportunities, the relatively short breeding season e.g., in Egypt the breeding season of dromedary camels is restricted to about three months (from late November to early March), The long gestation period of 13 months and late postpartum estrus. In addition poor pastoral management systems prevail in regions where camels are rised adversely affecting camel reproduction and productivity. Parasitic orchitis due to trypanosomaisis was the most harmful disease of camels. Brucellosis was also proved to cause chronic epididimo-orchitis, leading to persistence and spread of low reproductive potentials of these camels.

Studies carried out on slaughter material indicated that the female dromedaries are polyestrous breeders with marked fluctuation in ovarian activity during the breeding season. Ovulation in camles is non-spontaneous i.e., they are induced ovulators which means they normally only ovulate when mated. Graff follicles develop in one or both ovaries reaching a mature size of between 1.3-1.7 cm in diameter. This mature follicle stage lasts about 4-6 days in general and in the absence of mating or ovulation-inducing treatment, this mature follicle will regress and another follicle will start to develop. Development of an artificial insemination system, in combination with successful synchronization of estrus and induction of ovulation, are all necessary for applying selection programs and for more rapid genetic improvement in Egyptian camels. Previous studies have shown that an alpaca dummy could be used to collect semen from alpacas and therefore, a camel dummy was designed and constructed for easy and reliable semen collection from camels as designed by El- Hassanein Camel Dummy.

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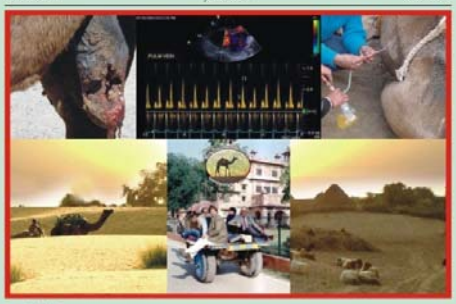

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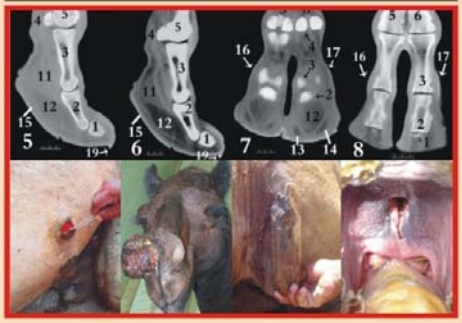

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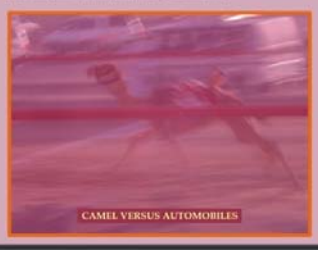

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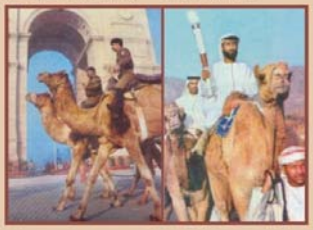

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The Second Conference of the International Society of Camelid Research and Development

Djerba, Tunisia: 11th-14th March, 2009

Introduction: The Arid Land Institute (IRA), Tunisia and International Society of Camelid Research and Development (ISOCARD), in cooperation with the Office of Livestock and Pasture (**OEP**), the Institution for Agricultural Research and Higher Education (**IRESA)** and the National Coordination Committee for Camel Research and development will organize the Second Conference of the International Society of Camelid Research and Development. The objectives of the conference are to promote research and publications quality in camelid, to encourage the exchange of information related to camelid and to elect members to serve on the Executive Council of ISOCARD for the next three years term

Venue: Hotel Yadis Djerba (Route Touristique, B.P.84, 4116 Midoun Djerba, Tunisia. www.maritim.com).

Language: English will be the official language of the conference.

Abstracts submission: Scientists working on camelid are invited to submit abstracts for oral or poster presentations. All abstracts will be reviewed by the Scientific Committee. Authors will be informed by **15th December 2008** whether the abstract is accepted for oral or poster presentation. The full text is requested before **1st March, 2009** to be published in the Journal of Camelid Sciences.
Deadline for submission of abstracts: **1st November 2008.** Abstracts should be sent by e-mail to Dr. Mohamed Hammadi (mohamed.hammadi@ira.rnrt.tn) in word document format. In the subject of the message, please, type 2nd Conference of ISOCARD and the name of presenting author.

For more information please contact:
Prof. Touhami Khorchani, Khorchani.touhami@ira.rnrt.tn

Or visit ISOCARD website: http://www.isocard.org/

Alpha-Vet Veterinary Ltd.

Veterinary medicines
Veterinary equipment
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Disinfectants
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Feed supplement
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Pharmaceutical Product Division

Our company distributes approximately 30 000 pharmaceutical products, instruments in connection with animal husbandry, pet food, pet accessories and cosmetics. In contribution with around 500 Hungarian and external suppliers we can provide our customers an extensive variety of products.

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On the basis of our permanent market researches we offer veterinary pharmaceutical products always with excellent quality at the best prise. Our suppliers need to meet the quality requirements determined by us and by our customers, just as permanent self control. We regularly analyze the veterinary pharmaceutical market in order to estimate the prospective consumer needs in due time thus we can meet the requirements and satisfy the expectations of our customers with providing top quality and wide range of products.

We are waiting our customers with courteous attendance and continual medical guidance in our central office's pharmacy with 2000 m² located in Székesfehérvár, in a modern environment complying with every technical requirement, open on weekdays from 7 a.m. to 8 p.m., on Saturday between 8 a.m. and 12 a.m. Our Customer Service Department is available on weekdays from 7 a.m. to 8 p.m. by phone, by fax, via e-mail or Skype.

Our computer network has been developed in a way that at the moment of the order, among others, we are able to inform about the stock quantity, the expected time of fulfilment, about the final sum of the invoice, about our discounts and our new products etc.

We believe that with the doubling of our warehouse area, the fourfold enlarged packaging room, the up-to-date workmanagement, the improved computer network system, respectively a prepared team of experts we can manage to raise the efficiency of our work in order to provide our customers an even more faster and accurate service.

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Logistics

One of the main objectives of Alpha-Vet Ltd. is to provide a high standard service. That's why we lay increased emphases to provide an excellent service in each section of the logistic processes. As a part of it we are continually extending our warehouses, respectively our motor pool and our colleagues regularly attend professional trainings.

Our extant warehouses are continually modernized and expanded. Currently we store pet food on approximately 5000 m2 and veterinary medicinal products on 1500 m2. Our site in Budapest was extended in 2007 with 1600 m2 warehouse, our store in Debrecen with a 400m² warehouse.

By means of our developed logistical structure it's realizable to reach each corner of Hungary weekly, in a form of organised rounds. At the present 24 trucks fulfil the daily delivery.

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Thank you for your visit !