The ICAR 2012 Satellite Meeting on Camelid Reproduction

3rd - 5th Aug

Vancouver, Canada

Eds: Dr. J. Juhasz, Dr. J.A. Skidmore, Dr. P. Nagy

PROGRAMME AND EXTENDED ABSTRACTS

ICAR 2012 SATELLITE MEETING ON CAMELID REPRODUCTION

Organized by

Camel Reproduction Centre

Dubai, United Arab Emirates

and

Emirates Industries for Camel Milk and Products (Camelicious)

Dubai, United Arab Emirates

In Cooperation with

International Veterinary Information Service (IVIS)

Ithaca New York USA

At

British Columbia Institute of Technology

Vancouver, Canada

ICAR 2012 SATELLITE MEETING ON CAMELID REPRODUCTION

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Preface

For many centuries Old and New world Camelids have made a big impact on human lives in many parts of the world but it is only recently that they have received particular scientific interest due to their potential as valuable racing, meat, milk and fibre producing animals.
This summer the 17th International Conference on Animal Reproduction (ICAR) is being organized in Vancouver, Canada bringing together many professionals and scientists from several continents so it was an ideal opportunity to organize the 2nd Satellite Meeting on Camelid Reproduction. Our aim is to draw the attention of the scientific community and international organizations to this field, to facilitate communication between colleagues, to support the development of Camelid research and to enhance the transfer of knowledge from science to practice.

In particular I would like to thank all our sponsors without whose support this meeting could not have been organized. They are:

Emirates Industries for Camel Milk and Products

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I am delighted that so many friends and colleagues were able to come to Vancouver and participate in this meeting and hopefully this Proceedings will be available through the IVIS website for those who could not attend.

The programme includes reviews and original research papers covering all aspects of reproduction, production and genetics in the camelid species and this proceedings contains the extended abstracts of the papers presented. It will also be published on the IVIS website www.ivis.org to enable fast distribution of information summarized at this meeting.

Looking forward to welcoming you to Vancouver.

Lulu Skidmore

Dr. J.A. (Lulu) Skidmore

Chair Camelid Satellite Meeting

List of speakers and presentations for the ICAR Camelid Reproduction Satellite Symposium

FRIDAY 3RD AUGUST 2012

8.30 – 9.30am	**REGISTRATION**	
9.30 – 9.45 am	Dr Lulu Skidmore **WELCOME**	
9.45 – 10.00 am	Prof WR Allen	**INTRODUCTION** The 20 year revolution in camelid reproduction: A cause for celebration
	SESSION 1 **Reproductive Physiology and pathology** **Chairman: Prof Amir Niasari-Naslaji**	
10.00 –10.15am	P. Nagy	Environmental factors affecting reproduction in dromedary camels
10.15-10.30am	Jane Vaughan	Identifaction of the ovulation –inducing factor in alpaca seminal plasma
10.30-10.45am	Marcello Ratto	Ovulation inducing factor (OIF) in the seminal plasma of llamas. Its physiological role in the hypothalamus-pituitary-gonadal axis
10.45-11.15am	**COFFEE/TEA**	**Chairman: Dr Alex Tinson**
11.15-11.30am	C. Rodriguez	Time interval between natural mating, vasectomized mating and GnRH on ovulation in alpacas
11.30-11.45am	D. Monaco	New treatments for the induction of ovulation in dromedary camel females: preliminary studies
11.45-12.00pm	K. Al- sobayil	Fertility response using two oestrus synchronization regimes in seasonally anestrous female dromedary camels
12.00-12.15pm	B.M. Manjunatha	Evaluation of hormonal protocols for synchronization of follicular wave and timed breeding in dromedary camels
12.15-12.30pm	R.O. Ramadan	Verification of ovarian hydro-bursitis syndrome in the dromedary and its surgical ablation
12.30-12.45pm	M.M. Waheed	Uterine histopathology as a tool for diagnosis of infertility in female camels
12.45-2.00pm	**LUNCH**	
	SESSION 2 **Semen and Artificial insemination** **Chair: Dr Jane Vaughan**	
2.00-2.15pm	J.A. Skidmore	The main challenges of embryo transfer and artificial insemination in dromedary camels
2.15-2.30pm	C.I Kutty	Collection, evaluation, processing and preservation of semen from dromedary camels
2.30-2.45pm	S. Purdy	Normal semen parameters in alpacas and correlation with pregnancy

2.45-3.00pm	W. Huanca	Use of seminal plasma to improve the pregnancy rate of reproductive technologies in alpacas: preliminary results
3.00-3.15pm	J. Gullaba	Cryopreservation of epididymal sperm in alpacas
3.15-3.30pm	P.W. Bravo	New Developments on artifical insemination of llamas and alpacas
3.30-4.00pm	**COFFEE/TEA**	
	SESSION 3 **Pregnancy and neonates** **Chair: Dr Jutka Juhasz**	
4.00 - 4.15pm	C. Donovan	Ovarian uterine and embryonic dynamics of early pregnancy in alpacas
4.15 - 4.30pm	G.B. Duffy	Circulating progesterone concentrations and progesterone receptor expression in the camel uterus during early pregnancy
4.30 - 4.45pm	M. Ferrer	Feasibility of ultrasonographic fetal gender determination in alpacas
4.45 – 5.00pm	Y. Picha	Induction of abortion in alpacas using cloprostenol or dinoprosttroethamine
5.00 – 5.15pm	S.S Dande	Investigations on clinical imunological and biochemical aspects of Indian neonatal dromedaries
7.30 – 10.30pm	**COCKTAIL PARTY** **at The TERMINAL CLUB, DOWNTOWN VANCOUVER.**	
SATURDAY 4TH AUGUST 2012		
	SESSION 4 **Embryo Transfer** **Chairman: Dr Marcelo Ratto**	
9.00 – 9.15am	Jane Vaughan	Embryo transfer in alpacas
9.15 – 9.30am	Marcelo Miragaya	Ovarian superovulation in South American camelids
9.30 – 9.45am	M.P. Horteloup	Effect of holding temperature on pregnancy rate of llama embryos
9.45- 10.00am	Julio Sumar	Reciprocal embryo transfer in alpacas and llamas
10.00- 10.15am	T. Huanca	Twin reciprocal embryo transfer between alpacas and llamas
10.15– 10.45am	**COFFEE /TEA**	**Chair: Dr Lulu Skidmore**
10.45- 11.00am	Tinson Alex	Factors affecting embryos recovery in dromedary camels: review of results over the last 20 years
11.00- 11.15am	A. Niasari - Naslaji	Interspecies embryo transfer: a suitable approach to conserve the Bactrian camel
11.15 – 11.30am	Tinson Alex	Observations on embryonic loss and abortion in racing camel (*Camelus dromedarius*) breeding programmes

11.30-11.45am	NisarWani	Cloning by somatic cell nuclear transfer in camels:cytoplast source influences the development of reconstructed embryos
	SESSION 5 Genetics	
11.45-12.00pm	Sanaullah	The Raigi Breed, newly found dromedary camel of the Pashtun region
12.00-12.15pm	N.Hedayat- Evrigh	Genetic characterization and differentiation of *Camelus dromedarius* in Iran
12.15 - 1.30pm	**LUNCH**	
	SESSION 6 **Production and management** **Chairman: Dr Peter Nagy**	
1.30 – 1.45pm	J. Juhasz	Mastitis control programme in a large scale camel dairy
1.45-2.00pm	L. Gupta	Nutritional strategies for improving growth, reproduction and milk production of dromedary camels in a changing climate
2.00-2.15pm	S. Wildeus	Effect of parasite management practices on fibre growth and quality in alpacas in the US Mid – Atlantic region
2.15 -2.45 pm	**COFFEE/TEA**	
2.45 -3.00pm	W. Brown	Improving breeding success for alpaca farmers in Southern Peruvian Highlands
3.00 -3.15pm	E. Abdel – Aal	Production and reproduction performance of camels herds under different production systems in Egypt
3.15 – 3.30pm	**Q & A SUMMING UP Arrangements for field trip**	
	FREE EVENING	
SUNDAY 5th AUGUST 2012 **Field trip to KENSINGTON PRAIRIE FARM** **Bus leaves ST REGIS HOTEL at 10.00am, RETURNS BY 3pm**		

The Organizers reserve the right to make changes to the order and content of this programme.

Day 1 and 2: Friday 3rd – 4thAugust 2012

Lectures presentations 8.30 am – 5.30 pm (Individual timings to be finalized)

Friday 3rd August 2012

Evening function at the Terminal Club, Vancouver.

Day 3: Sunday 5th August 2012

Field visit to KENSINGTON PRAIRIE FARM:

Kensington Prairie Farm is a beautiful farm in the Fraser Valley where Catherine Simpson and Jim Dales breed, raise and show Huacaya alpacas. They also market and sell a variety of high quality alpaca products from their on-farm store and produce artisanal honey and a small assortment of farm based food products.

Following a scenic one-hour drive through the beautiful Fraser Valley, tour participants will be welcomed to the farm by owner - Catherine Simpson and Canadian Llama & Alpaca Association President – Cathy Merkley. Tour guests will enjoy:

- Brief Canadian industry overview
- Huacaya & Suri Alpaca display
- Llama display & carting demonstration
- Hands-on alpaca fibre sorting and grading demonstration (Canadian Standards)
- Brief discussion of fibre processing/production options in Canada and around the world
- Alpaca/Llama Carding, Spinning & Weaving demonstrations
- Marketplace – Artisan & Imported Camelid Fibre Product Display

Approximate timings will be pick - up from St Regis Hotel 10.00am and return 2.30 - 3pm. Lunch will be included at Kensington Prairie Farm.

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هاتف : ٠٠٩٧١٤٢٢٨١٠٣٤, البريد الألكتروني : info@camelicious.ae

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Environmental factors affecting reproduction in dromedary camels *(Camelus dromedarius)*

Nagy P, Juhasz J

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Introduction

The dromedary camel is considered to be seasonally polyoestrous animal based on seasonal distribution of parturitions and surveys of ovaries from slaughterhouse specimens (Tibary and Anouassi, 1997). However, very few in vivo studies have focused on seasonal reproduction in the dromedary (Nagy et al., 2001; Vyas et al., 2004). Clearly, there is a lack of information on the occurrence and importance of seasonal reproduction in this species. Some authors suggest that seasonal distribution of conceptions is due to a decrease in libido of the males and to an increase in embryonic mortality during the summer months. In the same time, well-fed dromedaries show constant ovarian activity throughout the year (Tibary and Anouassi, 1997). Hence, it has been proposed that seasonality does not play a decisive role in female reproduction and it is primarily influenced by nutritional factors and not by photoperiod. However, we argue with this opinion and suggest that seasonal reproduction influenced by various external and internal factors is just as important in the reproduction of female dromedaries as it is in that of the male camel. In this paper, we aim to summarize existing information and demonstrate the importance of seasonality in the female dromedary camel.

The biological and the "official" breeding season

In the Northern Hemisphere, the breeding season usually extends from the autumn until the spring with some variation in different geographical areas (Tibary and Anouassi, 1997). In the Middle-East, the breeding season peaks between November to April. Our aim is to extend that period as much as possible in order to temper seasonal fluctuation in milk production. Our mating season extends from September until next June. In the last 5 seasons, 95 % (4701 of 4906) of the matings were performed during an 8 month period from October until May with peaks in December to January. In the racing industry, there is a pressure to

breed female dromedaries as early as possible, starting in August to have an advantage over calves born later. However, breedings early in the season are usually not successful. This discrepancy between the man-made, "official" and the biological breeding season underlines the need for research on this field.

The concept of endogenous circannual rhythm of reproduction

In other seasonal species, the annual reproductive pattern is the result of a circannual endogenous rhythm that is entrained by external environmental factors such as photoperiod, temperature, nutrition and body condition (Nagy et al. 2000). In ewes and in mares, under constant lighting condition or after the surgical removal of the pineal gland, the annual change in reproductive pattern is still manifested but it cannot be modified by external factors. The role of these factors is to synchronize the circannual endogenous rhythm of reproduction in order to guarantee the birth of the offspring at the optimal time of the year. We propose that similarly to other seasonal species, such a circannual endogenous rhythm of reproduction exists also in female dromedaries.

External and internal factors entraining the annual rhythm of reproduction

The photoperiod is known as the most important external factor influencing the endogenous reproductive rhythm. Seasonal species are classified either as short-day (sheep) or long-day (horse) breeders depending on whether the start of the breeding season coincides with decreasing or increasing daylight. Using such a classification, camels could be considered as short-day breeders. The stimulatory effect of light depends on several physiological factors such as photo-refractoriness, photoperiodic history and the existence of a photosensitive phase during the night (Nagy et al., 2000). It is assumed that changes in daily light are minimal in the Middle-East region and the ambient temperature can be extremely hot. For this reason, some authors suggested that the role of temperature is more important that that of the photoperiod influencing camel reproduction (Tibary and Anouassi, 1997; Marai et al., 2009). In fact, annual changes in photoperiod and temperature have a similar patter in the Middle-East (Latitude N25°) compared to the continental climate (Central Europe, Latitude 45°) but the changes are more moderate. The difference in daylight and average daily temperature between the summer and winter periods are 3 hours, 20-25 °C and 7.5 hours, 40-45 °C in the UAE and in Hungary, respectively. This fact suggests a similar relationship between photoperiod and ambient temperature in the Middle-East as in the continental climate. But neither the effect of photoperiod nor that of temperature on seasonal

reproduction has been convincingly demonstrated in camels (Vyas et al., 2008). On the other hand, nutrition and poor body condition have been shown to influence the start of the breeding season in dromedaries (Sghiri and Driancourt, 1999; Nagy et al., 2001).

Seasonal changes in reproductive activity of non-pregnant, non-lactating camels

During the non-breeding season, ovaries may become small, hard with only few small size follicles (>0.5 cm). On the other hand, during the breeding season, there is a constant follicular wave development. Some females show regular follicular activity throughout the year. However, the dominant follicles of the waves during the non-breeding season might not be functionally competent. Sghiri and Driancourt (1999) demonstrated that the steroidogenesis of early breeding season follicles was impaired compared to the peak of the season. For this reason, the beginning of the biological breeding seasons and readiness for conception cannot be unequivocally determined by morphological (ultrasonographic) examination in this species. In a previous study, we defined the 1st GnRH induced ovulation as the beginning of the biological breeding season (Nagy et al. 2001). Out of the 73 camels examined, 31 animals (42.5 %) were in anoestrus and 42 had active ovaries at the start of the season. The mean time of the first ovulation was on 24 December (± 6.79 days) in inactive camels. Age, body condition and lactation influenced ovarian activity at the beginning of the season (Sghiri and Driancourt, 1999; Nagy et al., 2001). Unfortunately, endocrinological studies on seasonality in female dromedaries are limited.

Interaction of season and ovarian activity in lactating camels: resumption of post-partum ovarian activity

We have monitored ovarian activity in lactating camels from May until October (Juhasz et al., 2004). During the first non-breeding season after parturition, several camels ceased follicular wave development and ovarian activity resumed only in late September, October. During the second non-breeding season, all lactating camels showed regular follicular development. This finding suggests a strong interaction between lactation and season in dromedaries at the beginning of lactation. In addition, we have observed that the time of resumption of post-partum ovarian activity is influenced by the time of parturition. Dromedaries delivering at the peak of the season (December to February) resume regular ovarian activity earlier then camels delivering at the beginning or at the end of the season (Nagy and Juhasz, unpublished).

Seasonal changes in fertility

Not only ovarian activity, but also conception rate shows seasonal variation. The overall conception rate per service (number of conceptions divided by the number of breedings) was 37.7 % on the farm in the last 5 breeding seasons. Conception rate increased from 20 % in September reaching a peak of 44 % in January and February, and then it declined again to 27 % in June. Parallel with this finding, the monthly distribution of conceptions (n=1615) shows a strong seasonal pattern with a distinct peak in January. In addition, we have preliminary data demonstrating seasonal difference in embryo recovery and pregnancy rate after superovulation and embryo transfer using the same donors in October to November and in April to May.

Seasonal changes in other, reproduction related parameters and in the hair coat

The length of gestation is significantly influenced by the month of mating. Similar finding has been reported in alpacas in New Zealand (Davis et al., 1997). The mean length of pregnancy is app. 2 weeks longer if mating is performed at the beginning of the season (389 days) compared to matings at the end of the season (374 days). Calf birth weight shows a similar seasonal change: calves born in December are app. 4 kg heavier (38.2 kg) than calves born at the end of the season (34 kg). In horses, the shedding of hair coat precedes the first ovulation of the year by app. 2 month (Ginther, 1992). In dromedaries, the natural shedding of hair coat occurs in the summer (i.e. 2-3 months before the breeding season). It was also demonstrated that the hair growth cycle shows a strong seasonal pattern with peak activity in the summer-autumn and inactivity in winter-spring (Ansari-Renani, 1998). It appears that dromedaries use the photoperiod rather than temperature as a signal for shedding of hair coat as it occurs after the summer solstice during the hottest period of the year. But, the association between reproduction and hair change has not been demonstrated in dromedary camels yet.

Conclusions

We conclude that reproduction in female dromedary camels is significantly affected by seasonal changes. But, the existence of an endogenous circannual rhythm and the interaction between photoperiod and temperature need to be elucidated in the future. With better understanding of seasonality in this species, new methods could be developed to extend the breeding season and to improve the overall fertility.

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Identification of the ovulation-inducing factor in alpaca seminal plasma

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The viscous nature of camelid seminal plasma hinders the handling and dilution of semen, but its composition is poorly understood. To date, non-specific enzymes have been used in various camelid species to assist with liquefying seminal plasma, but these enzymes have detrimental effects on sperm function and ultimately fertilisation ability due to their non-selective digestion of sperm and seminal plasma proteins (Morton et al., 2008). Dilution or removal of seminal plasma in other domestic livestock species may also improve or reduce sperm function (Kershaw-Young and Maxwell, 2011). Moreover, timing of artificial insemination in relation to induction of ovulation is also poorly defined. Therefore, the commercial viability of artificial insemination in alpacas, with or without cryopreservation, depends on a better understanding of the components and functions of seminal plasma.

During the separation and identification of camelid seminal plasma components, some major proteins have been isolated including enzymes, growth factors and an ovulation-inducing factor (OIF; Adams et al., 2005; Xilong et al., 2004). When OIF extracted from camelid seminal plasma is injected into camelid females with an ovarian follicle considered capable of ovulation, there is an LH surge that peaks 2-4 hours after administration and lasts approximately 6-8 hours. Ovulation is induced approximately 30 hours after treatment in a majority of females, and CL formation is first detected 2-3 days after treatment (Adams et al. 2005; Tanco et al., 2011).

Ovulation-inducing factor has also been isolated from other induced-ovulating species such as the rabbit and spontaneous ovulators such as cattle, horses and pigs indicating that it is a conserved constituent of mammalian seminal plasma (Bogle, et al., 2011; Silva et al., 2011).

Using one-dimensional gel electrophoresis, a 14 kDa protein (under reducing conditions) appears abundantly in seminal plasma (Kershaw-Young et al., 2012; Ratto et al., 2011). It has been isolated and identified as β-nerve growth factor by liquid chromatography

mass spectrometry, and shown to induce ovulation in female alpacas in a similar manner to the GnRH analogue buserelin and seminal plasma (Table 1.; Kershaw-Young et al., 2012).

Table 1: Follicle diameter before treatment, and corpus luteum (CL) diameter and plasma progesterone concentrations on Day 8 after treatment in female alpacas injected i.m. with 1 mL 0.9 % saline, 4 μg buserelin, 2 mL alpaca seminal plasma or 1 mg human β-nerve growth factor (β-NGF) in 1 mL 0.9 % saline (mean ± SEM; adapted from Kershaw-Young et al., 2012).

Treatment group	**Follicle diameter (mm)**	**Number of females ovulating**	**CL diameter (mm)**	**Progesterone* (ng/mL)**
Saline	8.0 ± 0.6^{a}	0/5	None present	0.12 ± 0.01^{a}
Buserelin	8.8 ± 0.7^{a}	4/5	9.3 ± 1.5^{a}	4.01 ± 0.90^{b}
Seminal plasma	8.2 ± 0.8^{a}	4/5	9.3 ± 1.3^{a}	2.44 ± 0.72^{b}
β-NGF	8.0 ± 1.2^{a}	4/5	10.3 ± 1.0^{a}	3.28 ± 0.68^{b}

[a,b]Values within a column with different superscript letters differ significantly ($P < 0.05$).

*Mean values for plasma progesterone concentrations do not include data from animals that did not ovulate, except in the case of saline-treated animals.

β-nerve growth factor is the most abundant protein in seminal plasma (Kershaw-Young et al., 2012), a finding that correlates well with findings on ovulation-inducing factor. On average, a llama ejaculate contains approximately 12 mg of ovulation-inducing factor (Tanco et al., 2011), the total protein content of alpaca seminal plasma is 40 mg/mL (Garnica et al., 1993) and if an average alpaca ejaculate is 1 mL (Vaughan et al., 2003), then the ovulation-inducing factor contributes 30 % of the total protein in camelid seminal plasma.

Nerve growth factors have been identified in the seminal plasma of camelid and non-camelid species, other than alpacas, and findings suggest that the source of β-nerve growth factor is likely to be from the accessory sex glands rather than the testes, and that β-nerve growth factor is likely to work at the level of the hypothalamo-pituitary axis, inducing ovulation by stimulating the secretion of LH (Kershaw-Young et al., 2012).

The identification of β-nerve growth factor as an abundant protein in alpaca seminal plasma, that induces ovulation in alpacas may assist with the development of protocols to induce ovulation.

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Ovulation inducing factor (OIF) in the seminal plasma of llamas: Its physiological role in the hypothalamus-pituitary-gonadal axis

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Introduction

Ovulation in mammals involves pulsatile release of GnRH from the medio-basal nuclei of the hypothalamus into the hypophyseal portal system with subsequent release of LH from the gondotropes of the anterior pituitary into the systemic circulation (Karsch, 1987). Elevated circulating concentrations of LH elicit a cascade of events within the mature follicle culminating in follicle wall rupture and evacuation of its fluid and cellular contents (Richards et al., 2002). The broad classification of species as either spontaneous or induced ovulators is based on the type of stimulus responsible for eliciting GnRH release from the hypothalamus (Baker and Baum, 2000). In spontaneously ovulating species (e.g., human, sheep, cattle, horse, pigs), release of GnRH from the hypothalamus is triggered when, in the absence of progesterone, systemic estradiol concentrations exceed a certain threshold (Knobil, 1980; Jaffe and Keys, 1974; Turzillo and Nett, 1999; Kelly et al., 1988). As a consequence of regularly occurring luteolysis and development of one or more estrogen-producing follicles, a preovulatory surge in circulating concentrations of LH occurs at regular intervals. In induced ovulators (e.g., rabbits, ferrets, cats, camelids), however, neural signals from copulatory stimulation trigger GnRH secretion from the hypothalamus, followed by the preovulatory release of LH from the pituitary (Baker and Baum, 2000). Similar to spontaneous ovulators, a surge in the circulating concentration of LH appears to be a prerequisite for ovulation in induced ovulators, but its occurrence is contingent upon copulatory stimuli; hence, ovulation is not a regular cyclic event.

The phenomenon of induced ovulation has been demonstrated in llamas (England et al., 1969), alpacas (Fernandez-Baca et al., 1970; San Martin et al., 1968), and Old World camelids (Chen et al., 1980), but very few studies have been conducted to determine the

factors responsible for eliciting ovulation in camelids. Also, in the only study of its kind in New World camelids (Bravo et al., 1990), ovulation induced by natural mating in llamas was associated with a rise in plasma LH concentration beginning within 15 minutes of mating. From these early studies, camelids have been classified as reflex ovulators because ovulations were only induced in the females after natural mating - a concept that has become accepted dogma.

Isolation and Purification of OIF

The existence of an ovulation-inducing factor (OIF) was first postulated in 1985 by Chinese scientists working with Bactrian camels (Chen et al., 1985; Xu et al., 1985); however, some weaknesses in experimental design limited the impact of the initial discovery. In a series of studies designed to test the hypothesis of the existence of OIF in South American camelids, we found that seminal plasma of llamas and alpacas induced ovulation via a systemic (not local) route by eliciting a preovulatory LH surge (reviewed in Adams et al., 2012). Results were consistent with a study in which alpaca seminal plasma stimulated LH secretion in vitro from a primary culture of rat pituitary cells (Paolicchi et al., 1999). The authors suggested that the putative ovulation-inducing factor in seminal plasma had GnRH-like activity but was not GnRH because its biological activity on rat pituitary cells was not suppressed when GnRH antibodies were added to the culture medium. Results of a more recent study confirm the effects of OIF on gonadotrophs of llamas and cattle (Bogle et al., 2012).

We have recently isolated and characterized the biologically active fraction of llama seminal plasma dubbed OIF as a robust protein, resistant to heat treatment and enzymatic digestion (Ratto el al., 2010). Further purification procedures have identified it as a 14kD protein molecule from the seminal plasma of llamas (Ratto et al., 2011).

Role of ovulation inducing factor (OIF) on the Hypothalamus-Pituitary axis

We have recently conducted two studies in our laboratory to determine if OIF induces LH secretion directly at the level of the pituitary or indirectly through an effect on the hypothalamus, and if its effect is modulated by the presence of ovarian steroids (estradiol). In the first study (Silva et al., 2011), llamas were pre-treated with a GnRH antagonist, Cetrorelix, and then treated with either GnRH or purified OIF. Neither ovulation nor a preovulatory LH surge occurred in the antagonist-treated llamas, suggesting that OIF induces LH secretion through stimulation of the hypothalamic GnRH neurons. The second study (Silva et al., 2012) was designed to characterize the effect of OIF on pituitary LH secretion in

ovariectomized llamas and to determine the effect of OIF on LH secretion in ovariectomized llamas pretreated with estradiol. LH secretion tended (P=0.08) to be lower in ovariectomized llamas than in the intact group. However, pre-treatment with estradiol partially restored LH release in OIF-treated ovariectomized llamas (P<0.05) to the values near those of intact OIF-treated llamas. We conclude that OIF elicits LH secretion by acting directly or indirectly on the GnRH hypothalamic neurons and that peripheral estradiol concentration partially modulates the effect of OIF on pituitary LH secretion

Acknowledgements

This study was supported by grants from the Chilean *National* Science and Technology Research *Council* (Fondecyt 11080141, Fondecyt 1120518) and the Natural Sciences and Engineering Research Council of Canada.

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Time interval between natural mating, vasectomized mating and GnRH on ovulation in alpacas

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Introduction

Alpacas and llamas are termed induce ovulators as copulation is needed to induce ovulation, thus it is more correct to say that they have follicular wave cycles lasting 12 to 16 days with no spontaneus corpus luteum (CL) formation, rather than oestrus cycles (Bravo and Sumar, 1989). This means that the timing for mating, artificial insemination or embryo transfer relies entirely on signs of oestrous behavior, which can be very variable and not clearly defined (Sumar et al., 1993; Sumar 1996). Nowadays, natural mating, LH, hCG, GnRH and vasectomized males are used to induce ovulation in alpacas and llamas for multiple purposes. In alpacas, the ovulation evaluated after necropsy has been detected as early as 26 hours after mating and 24 hours after hCG treatment (San Martin et al., 1968; Fernandez–Baca et al., 1970). In llamas, ovulation was detected by ultrasonography 27.2 hours after hCG treatment (Adam et al., 1992), 30 hours after GnRH treatment (Adam et al., 1992; Ratto et al., 2006) and 29.5 hours after mating (Ratto et al., 2006). Ultrasonography has the advantage of giving us a clear image and the ability to measure the size of the follicles, but the need for repeated transrectal manipulations might cause stress to the animal resulting in failure of ovulation and early embryo mortality.

The aim of this study was to compare the efficacy of natural mating, vasectomized mating and a combination of each natural treatment with the most commonly hormonal treatment (GnRH) to induce ovulation in female alpacas.

Materials and Methods

Experiments were carried out at the Embryo Transfer laboratory at San Antonio Research Station – SUMAC TARPUY in Puno, Peru. The experimental group consisted of mature non-pregnant female alpacas (n=43) with a body condition $\geq$ 2.8, twenty two entire males selected for their higher fertility rates in females and seven adult males vasectomized intrapelvically by laparoscopy (Bravo and Sumar, 1991). The ovaries of the females were examined once daily by transrectal ultrasonography using a B-mode scanner with a 7.5MHz

linear-array transducer (ALOKA 500D, Japan) and once the dominant follicle was observed, measuring between 7 to 13mm, they were assigned randomly to the following groups: (1) mated with an entire male (Mating, n=8); (2) mated with an entire male plus GnRH (Mating + GnRH, n=14) (Conceptal®, Intervet, 1.5ml IM); (3) mated with a vasectomized male (Vasectomized, n=4); (4) mated with a vasectomized male plus GnRH injection (Vasectomized + GnRH, n=10, 1.5 mL.IM), and (5) given a single injection of GnRH (GnRH, n=7). After ovulation induction, ultrasonographic examination was performed every 2 hours until disappearance of the follicle.

Collected data for follicle size and ovulation time were compared by ANOVA, followed by the Tukey post hoc test. Ovulation rate was compared by Chi-square test. The analysis was carried out by using GraphPad Prism 5.

Results

The size of the dominant follicle at the time of treatment was similar among the groups. Although there were no significant differences in ovulation rate between the groups, there was a tendency for higher ovulation rates in females mated with vasectomized males plus GnRH compared with the females mated with a vasectomized male alone (P>0.05). Similarly, there were no differences between groups in the time interval from treatment to ovulation (Table 1).

Table 1: Effect of mating with entire male or vasectomized male, with or without GnRH injection, or with GnRH alone on inducing ovulation in alpacas

	Mating (n=8)	**Mating +GnRH (n=14)**	**Vasectomized (n=4)**	**Vasectomized + GnRH (n=10)**	**GnRH (n=7)**	**P value**
Follicle size (mm)	9.4 ± 2.1	9.0 ± 1.6	10.8 ± 1.3	10.6 ± 2.3	10.2 ± 2.0	0.1871†
Ovulation rate	8/8 (100%)	13/14 (87%)	3/4 (75%)	9/10 (90%)	6/7 (86%)	P>0.05*
Timing ovulation (hr)	28 ± 2.4	26.9 ± 2.3	26.0	28.9 ± 3.0	26.8 ± 1.6	0.2180†

† Analysis by ANOVA
*Analysis by Chi square test

Discussion

The use of mating or gonadrotropin hormones to induce ovulation has been previously reported in some studies in alpacas. In previous studies, ovulation in alpacas was evidenced by the presence of a corpus luteum 3 days post copulation (Fernandez-Baca et al. 1970). More recently, however ovulation in alpacas has been evaluated by ultrasonography, but evaluation was carried out two days post ovulation induction (Ratto et al. 2011). To our knowledge, this is the first study where ovulation in alpacas was evaluated by ultrasonography every 2 hours from ovulation induction instead of every 4 hours, as previously described in llamas by Ratto et al. (2006). In this study the time from treatment to ovulation was not different between treatments, thus our data shows that the time interval between inducing ovulation and ovulation occurring in alpacas was on average 28 hours (range 24 to 32 hours). More precise information on the time interval from treatment to ovulation is presented here in comparison with previous studies where detection of ovulation was not so accurately recorded (Fernandez-Baca et al. 1970, Ratto et al. 2011).

The use of vasectomized males to induce ovulation gave rise to a lower, although not significantly lower, ovulation rate (75%) compared with the group where a vasectomized male plus GnRH (90%) was used. These results are similarly to those described by Sumar et al. (2010), who reported ovulation rates of 76.6% when using vasectomized males. However the poor response observed in our vasectomized group might be due to the reduced number of females mated with vasectomized males compared with those mated with a vasectomized male and injected with GnRH, so further studies using more animals should be performed to confirm this difference. Although seminal plasma alone has been reported to induce ovulation in llamas (Ratto et al., 2010) our results in the vasectomized group might suggest that not only is the presence of the seminal plasma needed to induce ovulation in alpacas but the presence of another protein or peptide produced from the testicle might be needed as well. Although ovulation rates among groups did not reach statistical differences, the use of vasectomized males might be a good and more economical method to induce ovulation in alpacas rather than using expensive hormones.

Conclusions

Ovulation time in alpacas occurs around 28±2.4 hours. Natural mating or using a vasectomized male for mating, even, without hormone injection provides sufficient stimuli to induce ovulation in embryo donor and recipient female alpacas and consequently, it might reduce the cost of synchronization for artificial insemination and embryo transfer purposes.

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New treatments for the induction of ovulation in dromedary camel females: preliminary studies

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Introduction

Ovulation in dromedary camels can be pharmacologically induced by Gonadotropin Releasing Hormone (GnRH) or by human Chorionic Gonadotropin (hCG) administration. Lecirelin is a nonapeptide, analogue of the natural GnRH, that has a glycine substituted by an ethylamide group at its terminal side. Lecirelin administration intramuscularly(i.m.) induced an LH surge in lamb ewes (Kaya et al., 2008) and, a single dose of Lecirelin (100 μg i.m.) produces results similar to hCG in the Jenny (Carluccio et al. 2007). Robbe et al. (2002) and Rizzo et al. (2011) observed that the epidural administration of Lecireline, compared with i.m. injection, is more effective for improving reproductive parameters in follicular cyst affected cows. The aims of this present study were: to understand if Lecirelin, intramuscularly administered, is effective for inducing ovulation in dromedary camels females and to verify if a known drug, Buserelin, could induce ovulation if administered epidurally.

Materials and Methods

The study was performed at National Research Centre on Camel, Bikaner, India, from the end of March to the beginning of May2011. Ten female, healthy, multiparous, non-pregnant, non-lactating camels, free from genital disorders and maintained under semi intensive system were used. Animals were restrained in sternal recumbent posture and Xylazine 0.3 mg/kg bw i.v. (Xylaxene®, Indian Immunologicals, Hyderabad, India) was administered for sedation. Ovarian follicles were first detected manually, and after by ultrasonography using an ultrasound device equipped with a 4-7.5 MHz probe (Vyas et al., 2004). If one follicle of minimum 10 mm in diameter was found, then the induction of ovulation was programmed. In Experiment 1 animals were injected with 100 μg of Lecirelin

i.m. (Dalmarelin®, Fatro, Italy) while in Experiment 2, 20 µg of Buserelin (Receptal®, Intervet, Italy) was injected in the first intercoccigeal space, with a 18G-38 mm needle (Skarda, 1996; Hall et al., 2001, Robbe et al., 2002). Ovulation was assessed by ultrasonography, and confirmed through serum progesterone determination 7 and 9 days post injection (Vyas et al., 2010). According to their diameters, follicles were divided in three categories: 10<**A**<13; 13≤**B**<20 mm; 20≤**C**≤30 mm; **D**>30 mm (Skidmore, 2000). For each category mean diameter ± Standard Deviation (SD) and ovulation rate (OR) were calculated.

Results

Eleven animals with follicles (n=11) were treated during Experiment 1 (Table 1). Three of them responded with the disappearance of the follicle and with an increase in serum progesterone (Table 2).

Table 1: Follicular categories and related ovulation rate after Lecirelin administration (i.m.)

Foll.Categories	**No. foll.**	**Mean diam. (mm) ± SD**	**No. foll. Ovul (%)**	**No. foll Not ovul**	**No. foll Lutenized**
10< A< 13 mm	0				
13≤ B < 20 mm	6*	15.6 ± 1.5	1 (16.6 %)	5	1
2≤ C ≤ 30 mm	3	20	1 (33 %)	2	0
D> 30 mm	1	33	1 (100 %)	0	0
Total	10		3 (27.3%)	7 (63.6 %)	1 (10%)

*****One follicle had luteinized and therefore was excluded from the data

The overall ovulation rate of B follicles was 16.6 % but one follicle from B category luteinized and was therefore excluded from analysis. Mean diameter of the C category was 20 mm and only one follicle ovulated (33% OR). The diameter of the D follicle was 33 mm and it positively responded to the Lecirelin treatment.

Table 2: Mean serum progesterone values (ng/ml) in female camels after Lecirelin administration

	Day 0 (GnRH)	**Day 7**	**Day 9**
Not Ovulated	0.44	0.48	0.49
Ovulated	0.59	3.13	7.13

Five animals with follicles (n=8) were injected in Experiment 2; four of them responded to the treatment (Tables 3 and 4).

Table 3: Follicular categories and ovulation rates after epidural administration of Buserelin

Foll. categories	No. foll	Mean diam. (mm) ± SD	No. foll Ovul (%)	No. foll Not ovul
10< A< 13 mm	0			
13≤ B < 20 mm	5	15.6± 1.9	4 (80 %)	1
2≤ C ≤ 30 mm	2	23 ± 4.2	2 (100 %)	0
D> 30 mm	1	45	1 (100 %)	0
Total	8		7 (87.5 %)	1 (12.5%)

One animal had one follicle on both ovaries while another had two follicles on the right ovary and another one (45 mm) on the left. Five of eight follicles belonged to B category, two to C, and only one to D. Mean diameter of B follicles was 15.6 mm±1.9 SD and 4 follicles ovulated, giving an ovulation rate of 80%. Mean diameter of C follicles was 23 mm ± 4.2 SD; both of them ovulated (100% OR) and the D follicle (45mm) positively responded to the epidural injection of Buserelin.

Table 4: Mean serum progesterone values (ng/ml) after epidural administration of Buserelin

	Day 0 (GnRH)	Day 7	Day 9
Not	0.54	0.50	0.70
Ovulated	0.55	3.28	6.71

One animal had one follicle on both ovaries while another had two follicles on the right ovary and another one (45 mm) on the left. Five of eight follicles belonged to B category, two to C, and only one to D. Mean diameter of B follicles was 15.6 mm±1.9 SD and 4 follicles ovulated, giving an ovulation rate of 80%. Mean diameter of C follicles was 23 mm ± 4.2 SD; both of them ovulated (100% OR) and the D follicle (45mm) positively responded to the epidural injection of Buserelin.

Discussion

Intramuscular administration of Lecirelin is effective in inducing ovulation in females camels but ovulation rates are low (16.6%) compared to those reported by Skidmore et al. (1996). These authors injected Buserelin or hCG (i.v) when dominant follicles measured between 10 and 19 mm in diameter, and reported ovulation rates of 81% and 67% respectively. In this experiment one follicle ≥20 mm and a 33 mm diameter follicle ovulated

in response to the treatment, whereas Skidmore et al. (1996) reported that follicles with a diameter > 30 mm will not ovulate in response to mating, Buserelin or hCG treatment. The reasons for these differences are not clear; it might be supposed that the intramuscular administration is not the proper route for Lecirelin administration or that follicles were not responsive, such hypothesis however need to be verified. The epidural administration of Buserelin was effective in inducing ovulation. Ovulation rates for B category (80%) were similar to those obtained by Skidmore et al. (1996) for follicles of similar diameter, whereas ovulation rates for Groups C and D were higher, but it should be noted that results cannot be compared to the work of Skidmore et al. (1996) in a realistic way due to the limited number of animals in this study. The mechanism by which the epidurally injected GnRH acts is unclear. The ovulatory response could be the result of the systemic diffusion of GnRH through lumbosacral foramina and/or the result of its diffusion in cerebrospinal fluid through the dura mater as happens with high doses of xylazine during caudal analgesia (Skarda et al., 1989). Moreover, GnRH and GnRH-receptors have been described within the mammalian spinal cord (Dolan et al., 2003) and it has been reported that GnRH might have a local effect acting through ovarian innervations (Ferruz et al.,1991). It could be supposed that interaction of the GnRH with local receptors improved its systemic effects thus enhancing the quality of the ovulatory stimulus. Detailed studies on the anatomy of the *cauda equina* in camels and the evaluation of distribution, pharmacokinetics and/or pharmacodynamics of drugs administered in the epidural space of dromedary camels are definitely required for a better understanding of the epidural injection mechanism of action. It would also be very interesting to assess the influence of different GnRH administration routes on the amplitude of preovulatory LH surge.

Conclusions

The GnRH-analogue Lecirelin could be used for the induction of ovulation in female dromedary camels. However further studies on administration routes are required before assessing the real efficacy of this drug as an ovulation inducing agent. The epidural administration of Buserelin acetate is effective for inducing ovulation in dromedary camels. This is the first report about the use of this route for the induction of ovulation in this species. More studies are required on the distribution and absorption of epidurally injected drugs.

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Fertility response using two estrus synchronization regimens in seasonally anestrous female dromedary camels

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Introduction

Reproductive activity in camels is primarily controlled by the ratio of daylight to dark, and estrus periods become more frequent as the days become shorter. It is notable in the Gassim region of Saudi Arabia, that fertility is highest and most efficient when she-camels are bred between November-April. However, the camel owners prefer their camels to calf in early fall but few of them give birth naturally in this period. This is why this study was carried out in September.

The estrous cycle in the female camel differs from that observed in most other farm animals (Skidmore, 2005) as it is an induced ovulator and thus only ovulates when mated. The estrous cycle is therefore characterized by the absence of the luteal phase and the existence of three follicular stages, namely follicular growth, the existence of mature follicles in ovaries (estrous period) and follicular atresia. These three phases last a total of 24-28 days (Tibary and Anouassi, 1997) within which the estrous period lasts up to 8 days. The estrous signs in the she-camel is characterized by it sitting down to be mounted by other females, restlessness, frequent salivation, swelling of the vulva, vaginal mucous discharge, frequent urination especially when the male comes close to her and finally, acceptance of mating by the male. In the last three decades, with the increased interest in artificial insemination and embryo transfer, estrous synchronization regimens have gained more attention of several research groups (Tibary and Anouassi, 1997). The progress in the methods of hormone extraction, purification and measurement of their concentrations in blood circulation has raised our understanding of the mechanisms regulating reproduction in farm animals (Combarnous and Anouassi, 1994).

It is well known that ovulation in female camels occurs after natural mating or hormonal induction with either: GnRH, LH or hCG. Administration of 0.5 – 1 ml GnRH (i.m. or i.v.) has been reported to induce ovulation 26-28 hours after injection (Musa et al., 1993) but it does depends on the size of the dominant follicle(s) at the time of hormone

injection. When she-camels with ovarian dominant follicles greater than 10 mm in diameter were given LH, the ovulation rate approached 90% (Tibary and Anouassi, 1997).

Understanding the hormonal changes throughout the estrous cycle of the she-camel helps in the application of different hormonal combinations to induce and synchronize estrus. As with other mammals, to synchronize estrus and ovulation in camels requires administration of specific hormone preparation(s) to stimulate ovarian follicular growth, development and ovulation. Equine Chorionic Gonadotrophin (eCG) has been used in she-camels, at a dose ranging between 1500-7000 IU, to stimulate follicular growth, to accelerate puberty, to shorten the calving interval or to induce estrus out of the breeding season (Elias et al.,1985; Homeida et al.,1991; Agarwal et al.,1993; Al-Sobayl, 2003 and 2008). Likewise, estrous synchronization in she-camels has been attempted by the administration of progesterone, or its analogues, either as daily injections or as a vaginal insertion of a PRID for a period of 10-20 days and injecting 1500-2000 IU eCG one day before or on the day of PRID removal (Tibary and Anouassi,1997).

The main objective of the present study was to meet the desire of camel breeders in the Gassim region who prefer their camels to calf very early. Therefore, we compared the effects of applying different hormone regimens on inducing and synchronizing estrus in dromedary she-camels.

Materials and Methods

Animals and Treatments

The present study was conducted with mature seasonally anestrous dromedary she-camels at the Veterinary and Agricultural Research Center, Al- Gassim University, Saudi Arabia during early Fall, 2008. Thirty mature 6-9 years old females were randomly divided into three equal groups (n = 10) as follows:

Group 1: A controlled internal drug release (PRID, 1.9 g progesterone) device was inserted into the vagina of each female for 12 days, and then each received an intramuscular injection of 2000 IU of eCG on the day of PRID removal.

Group 2: Females were injected with 1ml GnRH (GONAbreed®) on days 0 and 9, and 2ml prostaglandin $F_{2\alpha}$ (Estrumate ®) on day 7.

Group 3: Controls. Females in this group had no hormonal treatment and were kept together with a fertile male.

Ovarian follicular development in all treated females (Groups 1 and 2) was monitored by rectal ultrasonography, and natural mating (to one of three white mature fertile males) was

allowed when the follicle had reached ≥12 mm in diameter. Jugular vein blood samples were taken from all females in Group 1 on the day of device insertion, day 5 post insertion and on the day of device removal, and the serum decanted and stored until assayed for progesterone concentrations. Progesterone was measured using an Elisa Test (Human Test Kit, Germany) which was based on competitive interaction of progesterone and the hormone-enzyme conjugate for a limited number of immobilised anti-progesterone antibodies. The absorbance of calibrators and specimen was determined by using a Biotec Elisa microplate reader.

All animals were fed barley grains as a concentrate (3 kg /head/day) containing 8% crude protein and alfalfa hay with tap water *ad libitum*.

Statistical Analysis

Data were collected and statically analyzed. One way Analysis of Variance (ANOVA) was used to determine the differences in the independent variables among groups. Tukey's Honset Significant Difference was used to determine the multiple comparisons of means. The significant level was set at $P \leq 0.05$ and SAS software (SAS, 1996) was used to perform all statistical calculations.

Results and Discussion

The hormonally treated females in Groups 1 and 2 showed signs of estrus significantly earlier than control females ($p < 0.05$), with the percentage of animals showing estrus being 80, 40 and 0% in Groups 1, 2 and 3 respectively. Estrous behaviour started 30 h after device removal in Group 1 and 24 h after the second GnRH injection in Group 2. In Group 1 plasma progesterone profiles were 0.6, 3.8, and 0.7 on the day of device insertion, day 5 post insertion and on the day of device removal respectively.

Synchronizing female camels with progesterone (PRID) + eCG resulted in better estrous behaviour within a narrower time period, which enables camel breeders to breed their females without the need for continuous heat observation. After the PRID removal, clear estrous signs were observed (i.e. chasing and mounting other females, less appetite, aggregation, salivation, restlessness, swelling vulva, vaginal mucous discharge and receptivity to male) 30 h later. However, there was one major managerial problem with PRIDs as the surrounding females tended to nibble the free end (the blue string) of the PRID device and withdraw it from the vulva. This was overcome by the reducing the length of the string to 5cm so it couldn't be caught by the females.

At this time of the year, the end of the non-breeding season, the three males that were used were not 100% sexually active, so they did not breed all the females who showed signs of estrus. It is thought that the stimulation of sexual activity in male camels at the end of their non-breeding season is very important to increase the herd fertility when using estrus synchronization programs to synchronize the females.

Control females neither displayed estrus nor conceived. However, administration of GnRH/PG/GnRH (GPG; Group 2) caused moderate induction of estrus and resulted in 20% calving rates. However GPG did not work very well due to the fact that the first GnRH dose failed to induce ovulation and corpus luteum formation. The highest calving rate (40%) was achieved in Group 1 when PRIDs were followed by eCG injections. The physiological explanation for such an increase in pregnancy rate of the PRID + eCG versus GPG treated females was that eCG after progesterone enhanced follicular development by producing large follicle(s) (≥12mm in diameter). These results agree with those of Tibary and Anouassi (1997) who reported that synchronization of follicular development and ovulation is improved by administration of 1500 – 2000 i.u. eCG one day before PRID removal, or on the last day of progestogen treatment. In addition, Simpkin (1987) has shown that a 15-day progestogen treatment followed by the administration of 1500-2500 i.u. eCG guaranteed the presence of mature follicle(s) and subsequent ovulation (2-5 follicles, ≥12mm in diameter).

There were no significant differences between treatment groups in regard to gestation length (mean 378.3 day with a range of 370 - 384 days) and all females calved during the day. All the newborns were male except for one female, whose birth weight was only 34 kg compared with the male's 38.5 kg, and all were white in color similar to the bull sires. It is well known among camel breeders that some calves inherit their coat color from the bulls, which may be due to the dominance of the gene(s) responsible for this trait in the bull. In addition, camel breeders say that some males will only produce male offspring and others only females but these observations need further investigation to ascertain whether or not they comply with scientific fundamentals.

In conclusion, as the camel is so well adapted to harsh, dry conditions it is becoming an increasing important animal in desert environments. It is therefore very desirable to try and increase their reproductive performance and thus there is a need for inducing and synchronizing fertile estrus in she-camels early in the season. This study shows that synchronization of female dromedaries with progesterone (PRID), for 12 days followed by an injection of 2000 i.u. eCG (i.m) can improve the reproductive performance of the dromedary camel at the end of their seasonal non-reproductive period. The use of GnRH/PG/GnRH

however, does not appear to stimulate the whole ovarian system. There is still a lot of work to be done in this area.

Acknowledgment

I would like to express my deep thanks and appreciation to all those who help me to complete this research.

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Evaluation of hormonal protocol for synchronization of follicular wave and timed breeding in dromedary camels (*Camelus dromedarius*)

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Introduction

The ability to detect estrus efficiently and accurately is probably one of the most important factors to increase the reproductive efficiency in most farm animals including camels. The signs of estrus in dromedary camels do not always correlate with the ovarian follicular status (Skidmore et al., 1996) and therefore, estrus signs cannot be used reliably to decide the time of breeding. Several hormonal protocols have been developed in dairy cattle to breed them at a predetermined time rather than using detection of estrus. Hence, this study was conducted to assess the efficiency of a hormonal protocol for synchronization of follicular waves and timed breeding in dromedary camels during the breeding season.

Materials and Methods

This study was conducted at Royal Camel Corps, Royal Court Affairs, Muscat, Sultanate of Oman (latitude 23° 36' N: longitude 58° 37' E) during the peak breeding season (December to March). Camels were housed in pens isolated from males and were fed fresh green grass/dry fodder and had free access to water and mineralized salt lick blocks. The hormonal protocol used for synchronization of follicular wave was as follows: 100 µg of GnRH (GnRH-1; Cystorelin, Ceva Sante Animale, France) on Day 0, 500 µg of $PGF_{2\alpha}$ (PG-1; Estrumate, Schering-Plough Animal Health, Australia) on Day 7, GnRH (GnRH-2) on Day 10 and $PGF_{2\alpha}$ (PG-2) on Day 17.

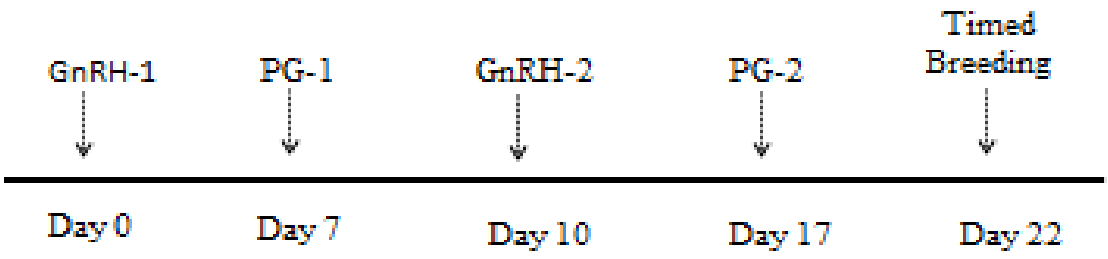
In Experiment 1, ovarian follicular dynamics were monitored in multiparous dromedary camels (n=72) by periodic ultrasonographic examinations (2 to 3 times/week). Ovulation was induced by intravenous administration of GnRH when an ovulatory size dominant follicle (DF; 11 to 18 mm) was present in the ovaries, which synchronized the follicular wave of these animals to a known stage of follicle development. The effectiveness of the hormonal protocol to synchronize follicular waves was evaluated by initiating the hormonal protocol at four different stages of follicular development which were as follows:

Group I, 4 - 7 mm; Group II, 7.1 - 11 mm; Group III, 11.1 - 18 mm and Group IV, 18.1 - 30 mm in diameter. Ultrasonographic evaluations were performed at 48 h after GnRH-1 to determine ovulatory response, at the time of GnRH-2 and 48 h later to determine the size of the largest follicle and subsequent ovulatory response and on days 12, 13 and 14 after GnRH-2 to determine the size of largest follicle. Ovulation was detected by the collapse/disappearance of the DF(s) 48 h after GnRH treatment, and confirmed by subsequent detection of a corpus luteum. In Experiment 2, this hormonal protocol was initiated at random stages of follicle development and animals (n=35) were bred by natural mating at a fixed time, 12 days after GnRH-2 (Fig. 1). The size of the largest follicle at the time of breeding, and its subsequent ovulation 48 h later were recorded, and pregnancy diagnosis was carried out on days 20 and 60 post breeding also by ultrasonography.

Statistical analyses

Between the groups the diameter of the DF at the time of GnRH treatment was analyzed by ANOVA with Post-hoc Tukey test and the ovulation rate was analyzed by a chi-square test. The level of significance set at $P \leq 0.05$.

Figure 1: Schedule of hormonal injections used for synchronization of follicular waves and timed breeding in dromedary camels.



Results

The mean size of the largest follicle at the time of GnRH-1 and GnRH-2 and the ovulation rate following GnRH-1 and GnRH-2, differed ($P<0.001$) between groups (Table 1). However, there was no variation in the mean size of the largest follicle on days 12, 13, and 14 after GnRH-2 among groups.

Table 1: Efficiency of hormonal protocol beginning at various stages of follicle development for synchronization of follicular wave in camels. (mean ±S.E.M)

Stages of follicle development	n	GnRH-1		GnRH-2		Days after GnRH-2 largest follicle size (mm)		
		Ovulation (%)	Largest follicle size (mm)	Ovulation (%)	Largest follicle size (mm)	12	13	14
Group I (4 - 7 mm)	15	0	5.8±0.3^{a}	66.7^{a}	17.6±0.6^{a}	13.8±0.4^{a}	15.0±0.4^{a}	16.4±0.4^{a}
Group II (7.1 - 11 mm)	23	21.7^{a}	9.0±0.2^{ab}	43.5^{b}	19.5±1.3^{ab}	13.9±0.3^{a}	14.9±0.3^{a}	16.6±0.3^{a}
Group III (11.1 - 18 mm)	19	100.0^{b}	14.0±0.4^{c}	100.0^{c}	12.0±0.2^{c}	14.0±0.4^{a}	15.4±0.4^{a}	17.0±0.4^{a}
Group IV (18.1 - 30 mm)	15	53.3^{c}	21.7±1.0^{d}	100.0^{cd}	12.3±0.3^{cd}	13.7±0.3^{a}	15.1±0.4^{a}	16.5±0.4^{a}
P value		*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.92*	*0.74*	*0.71*

Within a column, means and proportions with different superscripts differ.

In Experiment 2, a total of 25/35 camels conceived after breeding at a fixed time of 12 days after GnRH-2 (Table 2).

Table 2: Effect of a hormonal protocol initiated at random stages of follicle development for synchronization of follicular wave and timed breeding on pregnancy rate in dromedary camels.

No. of animals	Size of largest follicle at the time of breeding	Ovulation (%)	Pregnancy (%)	
			Day 20	Day 60
35	13.3 ± 0.4	94 (33/35)	71.4 (25/35)	65.7 (23/35)

Discussion

The present study showed that the hormonal protocol used to synchronize follicular waves and a timed breeding program was effective in a group of camels regardless of the stage of their follicle development at the time of treatment. Ovarian follicular development in dromedary camels occurs in a wave like pattern (Manjunatha et al., 2012a, 2012b) which is characterized by the emergence and synchronous growth of a cohort of follicles. One of these follicles continues to grow and becomes the future DF, while the others regress. When a growing DF reaches a diameter of 10 mm it acquires ovulatory ability, and its response to GnRH increases significantly when it reaches a diameter of 11 mm or more (Manjunatha et al., 2011). It was observed, in some circumstances, that when the follicles failed to ovulate (animals of Group I and II) after GnRH-1 treatment, they continued to grow and became dominant. On the other hand, following the ovulation or luteinization of the largest follicle (Groups II, III and IV) a new ovulating size DF was observed on the day of the GnRH-2 treatment. However, a new ovulating size DF was found 12 days after GnRH-2 treatment in all the animals of each group, irrespective of the size of the initial follicle at the start of the experiment. The size of the largest follicle on days 12, 13 and 14 after GnRH-2 did not differ between the groups.

The characteristics of the DF in dromedary camels appears to be unaffected by the progesterone secreted from the induced CL (Manjunatha et al., 2012b). However, progesterone secretion from an induced CL resulting from ovulation of a large follicle (>20 mm in diameter) or double ovulations was found to affect the growth of DFs in dromedary camels (unpublished data), hence, PG injections were included in the hormonal treatment mentioned in this study. Ovulation occurs at 31.7±0.6 h after ovulation inducing treatment (Manjunatha et al., 2011) and a new wave emerges two days after ovulation. The inter-wave interval period from the emergence of one wave to the emergence of the subsequent wave ranged from 11 to 21 days (16.36±0.37 days; Manjunatha et al., 2012a), therefore, the animals were bred 12 days after GnRH-2 treatment in the present study. The hormonal protocol used in this study for a timed breeding program resulted in a pregnancy rate of 65.70% after day 60 post breeding (Table 2). This pregnancy rate is similar to that found in our studies where the animals were bred by monitoring follicle development by ultrasonography, and mated when a dominant follicle reaches 13 to 18 mm in diameter.

In summary, this data demonstrates that synchronization of follicular waves and timed breeding can be carried out effectively using this hormonal protocol. This protocol could

have a major impact on managing reproduction of dromedary camels under field conditions, since it allows programmed breeding and eliminates the need for estrus detection.

Acknowledgements

The authors would like to thank His Excellency Nasser Bin Humood AlKindi, Engineer Mohamood Bin Badr Al Abri, Directorate General of Veterinary Services and Royal Camel Corps, Royal Court Affairs, Muscat, Sultanate of Oman, for providing the facilities and support to conduct this research.

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Verification of ovarian hydro-bursitis syndrome in the dromedary camels and its surgical ablation

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Introduction

Saudi Arabia is considered to have the fourth highest camel population in the Arabian world; with an estimated population of one million. According to our previous study (Ali et al, 1992; Al-Eknah, and Ali 2001), the characteristic features of the cyst-like structure that formed at the cranial part of the genital tract in female camels were that of an "infundibular cyst". The histopathology of the cyst wall consisted of fibrous connective tissue and smooth muscle, lined with low columnar epithelium. This was similar to the mucosal lining of the uterus which is also lined with columnar epithelium. Ahmed (1994) and Tibary and Anouassi (1997) described this cyst-like structure as "hydrobursitis". Recently, Ali, et al. (2011b) suggested that ovarian hydrobursitis syndrome is initially an inflammatory process and the accumulated bursal fluid originates partially from follicular fluid. More recently it has become evident that it is caused by fluid accumulation associated with Chlamydophila abortus infection (Ali et al., 2012). The objectives of this investigation were to verify the clinical nature of hydrobursitis in camels with emphasis on the outcome of surgical ablation.

Materials and Methods

A total of 600 female camels were examined at the Veterinary teaching Hospital of King Faisal University between September 2011 and March 2012. They were clinically normal and their general body condition was fair to good. Following routine rectal and vaginal examination, animals suspected of having hydrobursitis were subjected to an ultrasound examination using an Aloka SSD-500 machine attached to a 5-7 MHz linear array transducer (DesCoteatux, et al. (2010). Hydrobursitis was diagnosed in 41 animals (16.58%) aged between 6 - 14 years (average 10.8 years) and they belonged to 4 different breeds (Majaheem, Magatir, Higgins, Omani; see Table 1). Cysts smaller than 10 cm in diameter were treated by injection of 3000 i.u of hCG (Chorulon, Intervet International B.V*)* to

undergo spontaneous regression, whereas surgery was performed on 31 animals with cysts larger than 10 cm. Following surgery, the feedback obtained from 20 owners was recorded.

Table 1: Animals subjected to surgical interference

Age	Majahim	Magatir	Higgins	Omani
6 - >10	7	6		
10 – 14	10	5	2	1
Total	17	11	2	1

Surgical technique

Surgery was performed through the left flank at the dorsal to upper border of the rectus abdominis muscle, although occasionally the incision was made through the left para-inguinal region. Following laparotomy, the left hand was guided to the inner part of visceral peritoneum and then passed under the loops of intestine to hold the body of the uterus. The ovary is thus pulled to the operative field, but occasionally the cyst was held by a cord like structure connecting the uterine horn to the cyst and was therefore difficult to expose. The cysts were extracted to the operative field in toto, and those from the right and left sides were easily managed through the same surgical wound. Occasionally the cyst fluid was siphoned through the surgical wound first, and the cyst removed when it was reduced in size. Abdominal closure was achieved under the guidance of the operator's left hand to include the peritoneum with aponurosis of abdominal muscle in a figure of 8. This was anchored by another layer using Polyglagtin 910 (Vicryl) No 5 metric and the skin was closed with continuous mattress sutures re-enforced by Ford interlocking sutures. Antibiotics were given routinely for 5 days following surgery.

Following extraction of the cyst, one sample was taken for microbiology, another for chemistry, while the remainder of the cyst was fixed in 10% buffered formol saline for histopathology. Jugular vein blood samples were also collected in two tubes (EDTA and plain) from camels with cysts and control animals without cysts, for haematology and blood chemistry.

Results

Sixty percent of the cysts were located on the right bursa, 25% were on the left ovary and 15% occurred on both sides. Cysts of small and medium size could be diagnosed by rectal palpation. Cysts of more the 20 cm, however, created tension on the broad ligament, which was felt as a cord like structure, and fell beyond the range of the probe so were

therefore inaccessible. The ultrasonographic appearance of hydrobursitis ranged from an anechogenic to a homogeneous echo structure with lobulation of the content evident in some cases. In this study some of the cysts were delineated as clear dilation of the infundibulum and fallopian tubes with the bursa looking apparently normal.

The result of surgery was rewarding. The para-inguinal approach gave close inspection of both the ovaries but its healing rate was inferior. The dorsal to rectus abdominis method was therefore superior and both ovaries could be managed through the same wound. When the volume of the cyst was greater than 30 x 50 cm, however, it was advisable to release the tension within the cyst, by reducing its volume through aspiration of its contents. Out of the 31 animals operated upon, 20 owners responded to telephone feed-back with the following results: three animals had conceived after unilateral ovariectomy; three owners sold the camels shortly after wound healing and one animal died for reasons unrelated to the operation. The rest of the animals were still under observation.

The result of serum and cystic fluid samples in camels with cysts showed a slight elevation of AST, GGT and CK enzyme and a severe rise in BUN when compared with controls animals (Table 2) and the haematology picture showed severe leukocytosis and neutrophylia (Table 3; Nyang'ao, et al., 1997; Wernery, et al. 1999). The microbiological investigations isolated E. coli and Staph. Spp. in some cysts while other samples were sterile. The histopathology findings were that of papillary cystadenoma in some areas.

Table 2: Parameters in serum and cyst samples of animals (n=7) suffering from hydrobursitis compared with normal values

Parameter	**Serum**	**CYST**	**control**
ALB (G/DL)	3.5 ±1.6	2.56±3.6	4.9 - 10.2
ALP (U/L)	33.3±18.3	32.8±28.6	31 - 55
AST (U/L)	44±15	62.1±44.7	24.1 - 35.1
CA (MG/DL)	7.6 ±1.6	7.28±6.8	7.6 - 13.1
GGT (U/L)	116.6±17	153.3±250	9-17
TP (G/DL)	4.9±1.2	6.8±2.5	5.0 – 5.6
GLOB (G/DL)	1.3 ±0.9	0.25	0.45 - 0.65
BUN MG/DL	17 ±5.3	16.5±11.5	3.9 - 6.2
CK U/L	68.6 ±68.7	79.1±166	29.1-30.3
PHOS MG/DL	4.9±2.3	4.1 ±5.7	1.4 - 2.5
MG MG/DL	1.9±0.6	1.45 ±1.2	1.8-2.9

Table 3: Haematological values of the animals affected with hydrobursitis compared with normal values.

	MEAN	NORMAL
WBC	17.9±	**4 -12**
LYMPHOCYTES	5.6±	**2.5-7.5**
MONOCYTES	0.5±	**0 - 0.8**
NEUTROPHILS	11.7±	**0.6-4**
RBC	11.8±	**5-10**
HEMOGLOBIN	16.9±	**8-15**
PCV	34±	**24-46**
MCV	30.4±	**40-60**
MCH	14±	**11-17**
MCHC	47±	**30-36**

Discussion

The present study highlights the need to distinguish hydrobursitis from infundibular cysts in camels. In hydrobursitis there is an accumulation of fluid in the ovarian bursae and encapsulation of the ovary (Ali et al 2011a). In infundibular cyst syndrome the fluid is mainly confined to the infundibulum and fallopian tube, while the bursa and the ovary are normal (Ali et al 1992). It could be possible that hydrobursitis originates from infundibular cysts because the ovarian bursae and the fimbria of the fallopian tube are closely related to each other. Hydobursitis and infundibular cysts can be caused by many types of parasites such as Trypaanasoma evansi or micro-organisms such as A. hydrophila,(Ali et.al 1992;Ali et al. 2011a). Such micro-organisms might present themselves by changing the blood profile noted in this study.

Conclusion

This study shows that unilateral surgical ablation of the cyst and corresponding ovary could improve fertility rates as has also been reported by Tibary and Anouassi (2001) and Ali et al. (2011b). However further studies are needed to justify surgical interferences.

Acknowledgements

The work is supported by a grant from Deanship of Scientific Research King Faisal University. The Technical assistance of Abdallatif Sagre, Badre El Hamdan, and Osama I Alturky is highly appreciated.

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Uterine histopathology as a tool for diagnosis of infertility in female camels (*Camelus dromedarius*)

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Introduction

The reproductive efficiency of dromedary camels is generally considered to be low (Tibary et al., 2006). Establishing the cause of infertility relies on performing an extensive examination of the reproductive tract that includes a general physical examination, palpation and ultrasonography of the reproductive tract and evaluation of endometrial biopsy samples (Powers et al., 1990; Tibary, 2004). This paper was designed to study the causes of reproductive problems in female dromedaries with great emphasis on the uterine histopathlogic changes accompanying infertility.

Materials and Methods

One hundred infertile female dromedaries (5–18 years old) were used in this study during the period November to April in two successive years. Their previous breeding history was obtained and a complete gynecological examination of each female dromedary was performed. The uterine biopsy technique used in camels is similar to that used in mares (Powers et al, 1990). Biopsies were collected from the left horn, fixed in 10% formalin, stained with haematoxylin and eosin and processed for microscopic examination. The data was divided according to season and age of females and analyzed using SPSS program (2007).

Results

Clinical examination of the female camels revealed four infertility problems: pyometra, repeat breeder, endometritis and mucometra. Table 1. shows that the highest frequency of repeat breeders was found during Winter, whereas the number of endometritis cases was significantly higher during Autumn. Table 2. depicts a significantly higher percentage of pyometra cases in age groups B and C, however the number of endometritis cases was significantly higher in age group B and the number of repeat breeders was significantly higher in age groups A and B.

Table 1: Effect of season on causes of female camel infertility

Season	Pyometra	Repeat Breeder	Endometritis	Mucometra
Autumn(Nov.-Dec.)	26.47 (n=9)	17.86^{b} (n=5)	54.17 ^{a} (n=13)	28.57 (n=4)
Winter (Jan.-Feb.)	35.29(n=12)	60.71^{a} (n=17)	16.67 ^{b} (n=4)	35.71 (n=5)
Spring (Mar.-April)	38.24 (n=13)	21.43^{b} (n=6)	29.17 ^{b} (n=7)	35.71 (n=5)
All groups	34	28	24	14

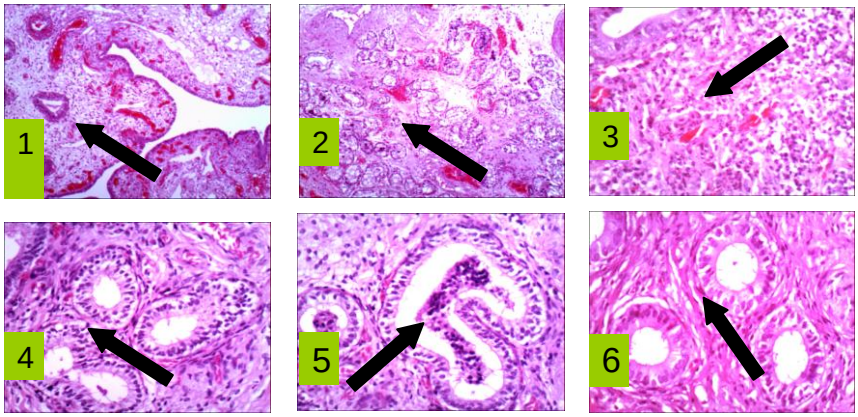
Percentages in the same column not sharing common superscript letters differ significantly P<0.05

Table 2: Effect of age on causes of female camel infertility

Age (years)	Pyometra	Repeat Breeder	Endometritis	Mucometra
A (4 – 6)	11.76^{a} (n=4)	35.71^{a} (n=10)	25.00^{b} (n=6)	21.43^{b} (n=3)
B (7 – 9)	29.41^{b} (n=10)	39.29^{a} (n=11)	37.50^{a} (n=9)	28.57^{ab} (n=4)
C (10 – 12)	41.18^{b} (n=14)	7.14^{b} (n=2)	20.83^{b} (n=5)	42.86^{a} (n=6)
D (12 <)	17.65^{a} (n=6)	17.86^{b} (n=5)	16.67^{b} (n=4)	7.14^{b}(n=1)
All groups	34	28	24	14

Percentages in the same column not sharing common superscript letters differ significantly P<0.05

Figure 1: (Grade 1A): uterus showing normal endometrium with normal uterine glands (arrow). X100; **Figure 2:** (Grade 1B): uterus showing few polymorphnuclear cells with increased secretory activity of uterine glands (arrow). X100; **Figure 3**: (Grade 2A): uterus showing extensive numbers of polymorphnuclear cells in lamina propria of stratum compactum (arrow). X400; **Figure 4:** (Grade 2B): uterus showing periglandular fibrosis (< 10 fibrocytic layers, arrow). X400; **Figure 5:** (Grade 2B): uterus showing cystic dilatation of uterine glands with secretions and desquamated cells (arrow). X400; **Figure 6:** (Grade 3A): uterus showing periglandular fibrosis (> 10 fibrocytic layers, arrow). X400.



The endometrial biopsies from the 24 camels (24%) with endometritis were examined histologically and assigned the following Grades (grading according to Powers et al., 1990): Grade 1A (figure1): was observed in one case (4.17%); Grade 1B (figure 2): in four cases (16.67%); Grade 2A (figure 3): in five cases (20.83%); Grade 2B (figures 4,5): in ten cases (41.67%); Grade 3A (figure 6): in four cases (16.67%).

Discussion

The uterus of female dromedaries could be the site of acquired abnormalities such as endometritis, pyometra and mucometra or be the reason for repeat breeding, all of which seriously affect female fertility (Tibary and Anouassi, 1997). In this study clinical diagnosis of infertile female dromedaries revealed similar abnormalities. The incidence of repeat breeders was 28% and were mostly recorded in Winter in the younger aged females. Higher percentages (76% & 40%) however, were reported in an earlier study by Tibary and Anouassi, (1997) and these occurred mainly during the Autumn. This could be due to the reduced follicular maturation that is observed at the beginning of the breeding season (Sghiri and Driancourt, 1999).

The percentage of pyometra cases in this study was much higher than the number of cases reported in earlier studies by Ribadu et al, (1991) where they ranged from 0.4 – 12.1%. Here the majority of pyometra cases were found during Spring, maybe because most parturitions occurred during Spring due to the influence of season on gestation length (Musa et al, 1993). In this study the greatest numbers of pyometra cases were recorded in age groups B and C, perhaps because at this age female dromedaries are at the peak of their reproductive performance with frequent mating, parturitions, postpartum complications and cervical adhesions.

The highest percentage of endometritis cases were reported during Autumn, probably because Autumn is the beginning of the breeding season when the cervical barrier is wide open and microorganisms can easily be carried into the uterine cavity as (Tibary and Anouassi, 1997). Endometritis was also most frequently recorded in age group B, mainly because the majority of female dromedaries in this age group are either at the puerperium period of first parturition or are mated during the first estrous period after parturition and these are times of high risk of uterine infection (Tibary and Anouassi, 1997). Endometrial biopsy samples from 24 dromedaries with clinical diagnosis of endometritis had abnormal histology findings in all but one case (4.17%), whereas in llamas there was a higher ratio (16.70%) of normal histology specimens in animals clinically diagnosed with endometritis (Powers et al, 1990). In this

study, the percentages of endometritis grades 1B (16.7%), 2A (20.8%), 2B (41.7%) and 3A (16.7%) found in camels were different from those reported in llamas which were 25.6%, 50.0%, 3.3% and 2.2%, respectively (Powers et al, 1990).

Four dromedaries in this study had uterine gland fibrosis severe enough to be classified as grade 3A. In mares with Grade-3 endometrium the pregnancy rate was 70.3%, but foal production rate was 35.1%, indicating appreciable resorption or abortion rate related to gland fibrosis (Shideler et al, 1982). In conclusion, uterine biopsy was readily performed without complications and could be considered as a good diagnostic tool of endometritis.

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The main challenges of artificial insemination and embryo transfer in the dromedary camel

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The reproductive efficiency of camels under their natural pastoral conditions is low partly due to their late age of reaching puberty, the short breeding season and a long gestation period of 13 months. The techniques of embryo transfer and artificial insemination however, provide the opportunity to produce more of the genetically superior animals, but they do pose certain challenges. The use of embryo transfer involves the necessity to superovulate the donors and synchronize the recipients and although good pregnancy rates are achieved with fresh embryos they are dramatically reduced after transfer of frozen/ thawed embryos. The development of AI in camels is complicated by the difficulty of collecting semen and the gelatinous nature of the semen produced, and to date there is still no reliable method for freezing camel semen.

Embryo transfer

Synchronization of donors and recipients

Pregnancy rates of 65 – 75% are now routinely achieved in camels after transfer of fresh Day 7 embryos into synchronized recipients that are on Day 5 or Day 6 after ovulation (McKinnon et al., 1994; Skidmore et al., 2002). However, synchronizing ovulation and follicular growth poses particular problems in camels, because they lack the cyclical corpus luteum of spontaneous ovulators. Synchronization of ovulation between donor and recipient camels can be achieved by selection of recipients from a random group of camels, where each recipient is examined 24 h after the donor is mated and those with a mature follicle in their ovaries are injected with GnRH (Skidmore et al., 2002). Alternatively, recipients are treated daily with progesterone-in-oil (100 mg/ day) for 10–15 days followed by administration of 1500–2500 i.u. eCG. The eCG treatment is planned for the day after the donor receives eCG and guarantees the presence of mature follicles in the recipient 24–48 h after the donor has ovulated (McKinnon et al, 1994). More recently two injections of GnRH given 14 days apart, with the second GnRH scheduled to be administered the day after the donor is mated, has given promising results with ovulation rates of over 80% after the second GnRH injection

(Skidmore, et al, 2009a; Nikjou et al, 2008). If the recipient should ovulate too early however the camel can be treated with meclofenamic acid to prolong the lifespan of the CL. Pregnancy rates of 80%, 60% and 70% have been achieved after embryo transfer into Day 8, 10 and 12 recipients maintained on 1g meclofenamic acid administered orally, from day 7 after ovulation to 7 days after embryo transfer. In addition, pregnancy rates of 56% have also been achieved when embryos have been transferred into recipients that have ovulated too late (ie are only at Day 3 or 4 after ovulation at the time of transfer) if they are maintained on daily injections of 75mg progesterone- in – oil/day (i.m.) from 2 days before embryo transfer until day 9 after ovulation (Skidmore et al., 2010).

Embryo transfer of fresh and frozen/thawed embryos

Pregnancy rates fall dramatically, to <40% after transfer of frozen /thawed embryos using conventional slow-freezing or vitrification techniques (Skidmore et al., 2004; 2005). Several factors could contribute to this reduced embryo viability post-thaw, including size of embryo, physical injuries caused by intracellular and extracellular ice formation, cryoprotectant toxicity, osmotic stress and chilling injuries. In addition, freezing and thawing can irreversibly disrupt the organization of an embryo's cytoskeleton and thereby reduce its ability to subsequently develop (Dobrinsky, 1996). Skidmore et al., (2009b) investigated the degree of cytoskeletal disruption suffered by cryopreserved camel embryos by freezing Day 6 and Day 7 embryos using slow cooling and vitrification methods. After thawing and rehydration the embryos were stained with 4,6-diamino-2-phenylindole dihydrochloride (DAPI) to identify dead cells, and labelled with Alexa Fluor 488-Phalloidinto assess cytoskeleton integrity. The results showed that vitrified–warmed embryos >300µm in diameter had a significantly higher percentage of dead cells compared with vitrified embryos ≤300µm, conventionally frozen embryos or controls. Cytoskeleton integrity was also affected by both freezing method and embryo diameter as all eight control embryos had a Grade I cytoskeleton, compared with only 2/24 (8.3%) frozen or vitrified embryos. Of the eight embryos with a Grade III cytoskeleton post-thaw, seven had been vitrified and six were larger (Day 7) embryos. These results indicated that while both slow-freezing and vitrification of camel embryos lead to cytoskeleton disruption and cell death, embryo quality is better preserved by slow-freezing.

Artifical insemination

AI is an important technique in several species to ensure rapid genetic progress, to enable more efficient use of superior males, to eliminate the need to transport live animals and reduce the spread of venereal diseases. Working with camel semen however produces many challenges due to the difficulty of collecting semen and its gelatinous nature makes handling and subsequent analysis difficult.

Collection and liquid storage of semen

The preferred method for collection of camel semen is with an artificial vagina (A.V) but males have to be trained to use an AV, which is difficult if they are used to natural mating. Camel semen is very viscous immediately after collection and as the spermatozoa are entrapped in this viscous seminal plasma they do not display forward progressive motility. This gel fraction cannot be separated off, thus estimating the motility is difficult and variable. It has been reported however, that semen will liquefy if left at room temperature for 20 – 30 min., but this varies between ejaculates. Various methods have been investigated to try and reduce this viscosity such as gentle pipetting, vortexing, centrifugation, density gradient centrifugation and various enzymatic methods, but only gentle pipetting of diluted semen was effective in reducing semen viscosity without compromising sperm motility or viability (Morton et al. 2008). However, treatment of camel semen with 0.05 mg/ml papain will also liquefy semen without detrimental effects to sperm acrosomal membranes. Moreover, the fertility after AI of fresh papain-treated sperm (30% pregnancy rate) did not significantly differ to that of fresh non-treated sperm (C. Kershaw-Young, K.M. Morton unpublished data) demonstrating the beneficial nature of papain treatment to liquefy camel semen.

More detailed studies are required to determine the most effective extender, but results to date show that the best extenders for liquid storage of semen are i) Green Buffer plus 20% egg yolk (v:v; 50% pregnancy rate (PR); Bravo et al., 2000) ii) an extender containing 11% lactose and 20% egg yolk (v:v; 50% PR; Anouassi et al., 1992) or iii) INRA – 96 plus (36% PR; Morton et al., 2010).

Number of spermatozoa and timing of insemination

The number of spermatozoa and timing of insemination also need refining in camels. Initial studies have shown that pregnancy rates of 50% can be achieved when 300 x 10^6 motile spermatozoa are inseminated (Bravo et al., 2000), although more recently insemination of 150 x 10^6 live spermatozoa into the uterine body, or just 80 x 10^6 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, 2006a).

Ovulation is known to occur 26 – 32 h after GnRH injection so to investigate the most appropriate time to inseminate, females were inseminated with 150 x 10^6 live spermatozoa either at the same time or 24 h after the GnRH injection. The results indicated that whereas 53% of camels conceived if they were inseminated 24 h after GnRH injection, only 36% conceived if they were inseminated at the same time as the GnRH injection (Skidmore and Billah, 2006b), suggesting that inseminating 24 h after GnRH injection is preferable.

Cooled Semen

Whereas several authors have investigated the efficacy of various extenders for cooling camel semen, only a few have carried out insemination trials. Deen et al, (2004) diluted split ejaculates with Tris based and Biociphos extenders, and found that none of the samples diluted in Biociphos exhibited any motility after 24 h at 4ºC, whereas 40% of spermatozoa diluted in Tris were motile after 24 h at 4ºC; however this was reduced to 10% by 96 h. Insemination trials have been carried out using cooled semen diluted in INRA (Morton et al., 2010) or Green Buffer (Bravo et al., 2000) but pregnancy rates are dramatically reduced to 20% and 25% respectively.

Supplementation with catalase

Medan et al, (2008) studied the effects of adding different concentrations of catalase enzyme to cooled dromedary camel semen (extended in tris-fructose- egg yolk extender) on semen quality during storage at 5ºC for 5 days. They found that the percentage of motile sperm was greatest, and of dead spermatozoa, sperm abnormalities and acrosomal damage was lowest, in cooled semen supplemented with 500IU/ml catalase. Moreover the conception rates of female camels inseminated with i) whole fresh, ii) extended, cooled, catalase–free and iii) extended, cooled semen supplemented with 500IU/ml catalase enzyme were 46.2, 22.2 and 37.5% respectively which would indicate that the addition of 500IU/ml catalase enzyme to semen extender can be used to prolong camel spermatozoa survival during storage at 5ºC.

Deep freezing of camel semen

The best preservation technique for any semen is deep freezing and storing in liquid nitrogen. In Bactrian camels Niasari – Naslaji (2007) recorded a post freeze/ thaw motility of 30% using SHOTOR diluent +6% glycerol, whereas pregnancy rates of an amazing 93%

have been reported after insemination of Bactrian camels with semen that had been frozen in a diluents containing 12% sucrose +7% glycerol by Chen et al, (1990). In dromedary camels Morton et al., (unpublished data) recorded a post thaw motility of 35% using a combination of Green and Clear Buffers (IMV, L'Aigle, France) but this was reduced to 0% after 3h post thaw. Other studies were carried out comparing packaging in 0.5ml straws to freezing in pellets and results showed that pellet frozen semen showed higher 0h and 3h post thaw motility, sperm membrane integrity and sperm viability compared with sperm frozen in straws although no pregnancy results were reported. Further work is being carried out using alternative extenders and cryoprotectants, such as INRA – 96 and amides, to try and improve post thaw motility so that acceptable pregnancy rates can be achieved.

These results show that embryo transfer and artificial insemination can be used to improve the reproductive efficiency of camels but more work is needed to establish reliable protocols for freezing and thawing their embryos and semen so that better pregnancy rates can be achieved.

Acknowledgments

The authors are grateful to Dr KM Morton, Dr C Kershaw-Young and Dr E Crichton for their invaluable help with the semen projects carried out at the Camel Reproduction Centre.

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Collection, evaluation, processing and preservation of semen from Dromedary camels (*Camelus Dromedarius*)

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Introduction

Unlike with other farm animals, artificial insemination (AI) is not popular in camels owing to the difficulties in collection (El-Hassanein, 2002) and preservation of semen, and poor success of AI especially with frozen semen (Tibary & Anouassi, 1997). Even though research studies are being carried out, there are certain problems in performing AI as a routine tool for breeding camels. In this context, research trials were initiated on semen preservation and AI in camels at the newly established Centre for Artificial Insemination and Embryo transfer under Abu Dhabi Food Control Authority. Preliminary studies were carried out in collaboration with a private farm to develop techniques such as semen collection, quality evaluation and processing for preservation of camel semen.

Materials and Methods

The study was conducted using nine adult male camels maintained under ideal management conditions on a private farm situated in Al Saad, 60 km from the Center. The camels were trained for semen collection using a modified bovine Artificial Vagina (AV) (Bravo et al. 2000, El-Hassanein et al. 2004, Tibary & Anouassi, 1997), and a female camel as the mount. The study started in November 2010 and, after 3-5 weeks of training, semen was collected at regular 3-7 days intervals until the middle of May 2011. Collected semen samples were subjected to quality evaluations where the volume, concentration, motility, proportion of dead sperm and sperm abnormalities were recorded.

Samples of optimum quality and quantity were diluted 1:2 to 1:4 (semen:extender) ratios depending upon sperm concentration, (El-Hassanein, 2006, Tibary & Anouassi, 1997, Wani et al., 2008), with the commercially available extender Triladyl, or Tris based diluents with 20 % Egg-yolk plus Gentamycin (50 µg /ml) added (Morton et al., 2009, Valliancourt et

al., 1993). No chemical agents or enzymes were used to achieve liquefaction, but gentle agitation with a plastic straw was found to be useful for mixing the viscous semen with the extender. Extended semen samples were put in a water bath and placed in a refrigerator and sperm motility was assessed at daily intervals.

Other semen samples with at least 70 % initial motility in Triladyl extender were loaded into 0.5 ml straws and frozen in liquid nitrogen vapour for 7-8 minutes, after which they were plunged into Liquid Nitrogen (-196˚C). These straws were subsequently thawed in a water bath at 37 ˚C for 1 minute and post-thaw motility was assessed.

Nine female camels with a mature follicle, of between 1.3 - 2.0 cm in diameter, were inseminated with fresh, extended semen after hormonal induction of ovulation (Tibary & Anouassi, 1997). The success rates of these inseminations were determined by behavioral signs such as "tail cocking" 15 days after insemination, and ultrasound scanning 25 days after insemination. Qualitative and quantitative data from the observations and semen studies were recorded, analyzed and the findings described.

Results and Discussion

Male camels were easily trained for semen collection although they showed varying levels of interest in serving into the AV. Throughout the breeding season a total of 178 semen samples were collected from 197 attempts using a bovine artificial vagina with minor modifications, but without an imitation cervix (Skidmore 2004) or special inner liners.

Reaction time (Deen et al., 2005) before mounting was less than 30 seconds and mean duration of mating was 5.08 +/- 0.14 minutes. The volume of semen ranged from 0.2 ml to 16 ml (mean 4.5+/-0.22 ml), which was similar to that reported by Wani et al., (2008). The color of the majority of the ejaculates varied between samples from grayish white to white as described by Skidmore, (2004) and Tibary & Anouassi, (1997), but occasionally it was yellow, dark brown or dark grey in color attributable to unusual contents or contamination. The larger volume samples however were whiter, homogenous, viscous and sperm rich. There was frequent contamination of semen with extraneous particles (21 %) and the occurrence was higher with increased mating duration and number of interruptions. High numbers of desquamated cells were also a common occurrence (33 %) in camel semen.

Almost 50 % of the samples were rich in spermatozoa and sperm concentration ranged from 1 - 1640 x 10^{6}/ ml (mean 364.66 x 10^{6}+/- 34.51) which was similar to that reported by El-Hassanein et al. (2004). The initial oscillatory motility of the semen was graded before extension of the ejaculates and was expressed in terms of symbols '+' to

'++++'. The results yielded "++++" grading for 33% of the samples. Oscillatory type movement and progressive forward motility recorded within 5 minutes of extension was 68.85 (+/- 2.35) % and 54.50 (+/- 2.36) % respectively, which increased to 74.2 (+/- 2.58) % and 57.93 (+/- 2.33) %, respectively, after 20-30 minutes of extension. Tibary & Anouassi (1997) reported a similar improvement in motility a few minutes after extension. The number of semen samples maintaining at least 50 % progressive motility after storage at 4˚C for 24, 48 and 72 h was 46 %, 23% and 10 % respectively. These results are similar to the mean progressive motility of around 40 % after 24 hours of storage at 4 ˚C reported by Morton et. al. (2009) and Wani et al., (2008).

The proportion of dead spermatozoa in these samples was 14.95 (+/- 0.95) %, which is less than that reported by El-Hassanein (2002) for semen collected using an AV. The number of dead spermatozoa was less in the less viscous compared with the highly viscous samples. Sperm abnormalities observed included detached heads (8.34 +/- 0.71 %), bent tails (6.7 +/- 0.71 %), structural defects of head (3.3 +/- 0.81 %), coiled mid pieces (3.15 +/- 0.31 %) and broken tails (1.4 +/- 0.20 %). Protoplasmic droplets were negligibly low in these samples unlike the frequent occurrence of protoplasmic droplets reported by El-Hassanein et al., (2004) and Tibary&Anouassi ,(1997).

Freezing of semen samples using Triladyl extender resulted in post thaw motility of ≥40 % in 34 % of the freezing attempts, which is lower than the figure reported by El-Hassanein, (2006). Only 4 samples yielded post thaw motility of ≥50% and all the samples showed a drastic reduction in post thaw motility after 3 months of storage; the reasons for which need to be investigated.

Of the nine camels inseminated with fresh semen, 5 showed behavioral signs of conception ("tail cocking") at 15 and 20 days after insemination and two were confirmed pregnant by ultrasound scanning. Further ultrasound examinations followed the development of these pregnancies for 45 days of gestation, but the growth rate was unsatisfactory beyond 30 days, and the conceptus had completely disappeared by 60 days of gestation. These inseminations were carried out during the beginning of the season (December) for various management related reasons and no AIs could be performed thereafter due to non-availability of animals.

Conclusion

Training and semen collection from camels using a modified bovine AV was accomplished in this study and confidence was gained in evaluation and processing of camel

semen. Problems associated with viscosity were overcome by physical means and trials were carried out with fresh, chilled and frozen semen. More studies are planned in the coming season to try and improve collection techniques, compare different extenders, improve motility of chilled and frozen/thawed semen, and more trials will be performed to try and improve pregnancy rates after insemination of fresh, extended, chilled and frozen/thawed semen.

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Normal semen parameters in Alpacas and correlation with pregnancy

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Introduction

The purpose of this study was to gather information regarding semen characteristics in 20 male alpacas and to examine the correlation of these parameters with pregnancy rate in some of these animals. Eleven of the 20 males were evaluated 4 or more times. Semen samples from study males were collected using an artificial vagina mounted in a breeding phantom, and also from the cranial vagina of live females immediately after the male dismounted. The postcoital semen sample analyses were correlated with pregnancy results as determined by transrectal ultrasound examination of the study females. Semen parameters evaluated for all samples included: semen volume (phantom breedings only), semen viscosity, estimated spermatozoa motility and concentration from an immediate post collection sample, spermatozoa live/dead percentage and spermatozoa morphology from eosin-nigrosin stained slides. If there was sufficient volume of sample, sperm concentration measured by Neubauer haemocytometer after semen liquefaction at room temperature for 24 hours, was also recorded. The ability to collect males using the breeding dummy was inconsistent. A wide variation in semen parameters was found in individual males, as well as between males, collected via the breeding phantom and after live breeding. Sperm motility, live/dead percentage, normal morphology percentage and concentration were evaluated with respect to pregnancies achieved in the study females. No direct correlation for any of these parameters with establishment of pregnancy was found in these males and females.

Materials and Methods

In this study 20 adult Huacaya male alpacas ranging in age from 2.5 - 11 years of age (mean age of 5.4 years) were used. They were split in approximately half with respect to previous breeding experience. All 20 of the males were used for semen collection and evaluation using the breeding phantom and an artificial vagina (AV) designed for sheep

(Agtech Inc., Manhattan, KS) and 8 of these males were used for natural service to correlate semen parameters from post-breeding collections with achieved pregnancies.

The 8 study females ranged from 3 - 11 years of age (mean age of 7.6 years) with 0 - 9 previous crias (mean of 3). The females were used for natural service breedings and for collecting postcoital semen samples (n= 24), which were then evaluated with respect as to whether or not a pregnancy was achieved. The females were also sometimes used as teasers for the males to increase libido during semen collections using the breeding phantom. The breeding phantom was constructed using a foam deer archery target, foam padding, and a tanned alpaca hide cover (Wiggin and Purdy, 2008). A section of the posterior of the archery target was hollowed out and a PVC pipe was placed inside to hold the artificial vagina. The AV apparatus was filled with water at approximately 45 °C and then pressurized using a rubber bulb until firm. It was then kept at the required temperature of approximately 40 to 45°C by either wrapping in an electric heating pad or in heated gel packs and inserted into the phantom. No AV lubricant or simulated cervix was used. The males varied widely in the amount of effort and time required to train them to mount the phantom and ejaculate into the AV. If the male did not commence orgling and mounting the phantom soon after introduction, a receptive female was used to attempt to stimulate libido. The male was allowed to mount the receptive female right next to the phantom until she assumed the normal sternally recumbent (kushed) breeding position. At this point the male was moved off the live female and onto the phantom. Usually after 1-2 trials using this technique, the male would learn to mount the phantom immediately upon introduction without the aid of a receptive female. If males were not mounting or ejaculating into the AV after a minimum of five attempts they were removed from the trial. Experienced males performed better with respect to mounting the breeding phantom and ejaculating into the AV.

Males were collected 1-3 times per week and were allowed to breed for 15-20 minutes or until they voluntarily dismounted and would not remount. The copulation time was limited to 20 minutes to prevent a possible decrease in semen viability. Once copulation ceased the AV was immediately removed from the phantom and brought inside. The semen sample was driven into the collection tube using centrifugal force and then the collection tube was removed and immediately placed into an incubator preheated to 37 °C until analysis was complete.

The postcoital semen samples were analyzed to evaluate any possible correlation between semen parameters and achieved pregnancies. These samples were obtained by inserting a plastic speculum into the vagina of the restrained, most often sternally recumbent, female

immediately post copulation. Gentle scooping of the speculum along the ventrum of the vagina near the cervix permitted a semen sample to be easily obtained and each sample was stored in an incubator at 37 °C until analysis was complete. Both the semen collected from the phantom breedings and the postcoital samples were analyzed for the same parameters using the same methodology. Semen viscosity (either height of semen column at breaking point or visually estimated as low, medium, or high), estimated percentage of motile spermatozoa, estimated and /or actual spermatozoa concentration, spermatozoa morphology, and percentage of live spermatozoa were all evaluated. The only parameter assessed for the phantom breedings that was not available for the postcoital samples was volume of the ejaculate, since the sample obtained via the speculum was only that which overflowed into, or was carried into, the vagina as the male dismounted.

Results

The results are summarized in Table 1. There was no effect noted on spermatozoa motility or live spermatozoa percentage with respect to photoperiod for any samples. These two parameters and all others measured were not consistent for individual males or among the 20 males analyzed. Of 101 total mounts on the phantom there were only 38 successful collections (38%). Out of the 45 collections in which sperm motility percentages were recorded, only 15 (33%) were successful with a motility percentage greater than zero. Semen viscosity was variable with higher sperm activity percentages noted in high viscosity samples compared with low viscosity samples. Spermatozoa morphology was extremely variable in the study animals but, on average, approximately 70% (range 30-90%) of the spermatozoa were morphologically normal per ejaculate. Cytoplasmic droplets on the sperm midpiece (indicative of sperm immaturity in other species) are normal in alpacas based on 47 collections of 10 males. Morphological data in this study indicated that proximal droplets averaged 8%, and distal droplets averaged 2%. Unpublished data from alpacas in the southern Peruvian highlands (Szymkowicz and Purdy, 2012) also showed spermatozoa with cytoplasmic droplets to be common. Midpiece defects and head abnormalities each accounted for 4% of abnormalities and approximately 11% of the sperm in each ejaculate had some sort of tail abnormality. This latter defect was the most common defect in the study animals. Sperm motility, live/dead percentage, normal morphology percentage and concentration were evaluated with respect to pregnancies achieved in the study females. No direct correlation for any of these parameters to pregnancy was found in the study males and females.

Table 1: Semen parameters of alpaca semen collected by artificial vagina (AV) and from the cranial vagina (CV) post coitus

Semen Parameter	Value (range or average) AV and CV
Sperm concentration	10-800 x 10^6 sperm /ml
Sperm activity	0-85%
Live spermatozoa	65-99%
Semen viscosity	10-80 mm semen thread break length
Sperm morphology	
Normal	30 - 90%
Cytoplasmic droplets - proximal	8%
- distal	2%
Midpiece defects	4%
Head abnormalities	4%
Tail abnormalities	11%

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Use of seminal plasma to improve the pregnancy rate of reproductive technologies in alpacas (*Vicugna pacos*): preliminary results

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Introduction

The development of reproductive technologies in alpacas such as artificial insemination and embryo transfer has increased in recent years aimed at increasing the number of genetically superior animals produced in breeding programs so that the quality of alpaca fiber used in the textile industry is improved. Alpacas, like all camelids are induced ovulators which means mating is required to induce ovulation (San Martin et al 1968). Ovulation in alpacas that are to be artificially inseminated or used as recipients in an embryo transfer programme, can be induced by intramuscular (i.m.) injection of GnRH analogues, however the presence of an ovulating inducting factor (OIF) in the seminal plasma of alpacas has been reported by Rios (1989) and later confirmed in alpacas and llamas by Adams et al (2005). Seminal plasma can, therefore, also be used as an alternative for inducing ovulation with subsequent development of a corpus luteum (CL) capable of maintaining pregnancy. The objectives of this study was to induce ovulation with seminal plasma and evaluate the effect on pregnancy rate in alpacas either inseminated with fresh semen (Exp. 1) or used as recipients in an embryo transfer programme (Exp.2).

Materials and Methods.

A total of 108 adult alpaca females of four to six years of age and with an average body weight of 63.7 ± 2.6 kg were used in this study. Two experiments were designed to evaluate the effect of inducing ovulation with seminal plasma in alpacas that were either i) inseminated with fresh semen or ii) used as embryo transfer recipients.

Semen from 10 adult male alpacas was collected one month before the experiment with an artificial vagina and diluted 1:1 (v/v) with phosphate buffered saline (PBS, Gibco, New York, USA). The diluted semen was centrifuged for 30 minutes at 1200 g and the

supernatant decanted and examined for the absence of sperm, then centrifuged again for 20 minutes at 1200 g. The seminal plasma was then stored at -80 ºC until used in the experiments.

Experiment 1: A total of 84 adult lactating female alpacas, that had had an uncomplicated calving more that 15 days previously, were examined daily by transrectal ultrasonography using an Aloka SSD500 scanner with a 7.5 MHz linear-array transducer. When a dominant follicle ≥ 7mm was detected in their ovaries they were assigned randomly to one of two groups and induced to ovulate with either i) 0.04 mg of GnRH (Acetate of Buserelin; Conceptal, Intervet, Ate, Peru; n=42) or ii) 1 ml of seminal plasma (n=42), injected intramuscularly (i.m.). Semen for insemination was collected by using an artificial vagina inserted into a dummy. It was then evaluated for spermatozoa concentration and motility before being diluted 1:3 (v:v) in an extender containing 0.3 % Bovine serum albumin (BSA) and 0.3 % Glucose. Each female was subsequently inseminated with 1.0 ml of fresh semen (with a concentration of 90 – 110 x 10^6spermatozoa/ml and > 60 % motility) by the recto-cervical technique at 28 ± 0.9 hours post induction of ovulation (Day 0).

Experiment 2: A total of 24 adult lactating female alpacas with a good body condition score and that had had an uncomplicated calving more than 20 days previously were examined daily by transrectal ultrasonography. When a dominant follicle ≥ 7 mm was detected in their ovaries they were assigned randomly to two ovulation groups i) GnRH (n=11) or ii) seminal plasma (n=13) injected i.m. (Day 0) as described above, and were used as recipients in an embryo transfer programme. The alpaca recipients were induced to ovulate the same day that the donor was mated so that they were exactly synchronized with the donor. Alpaca donors that had been stimulated with FSH, according to the protocol described by Huanca et al (2009), were mated and blastocyst stage embryos recovered on Day 7 after ovulation. Embryos of similar quality were assigned to both groups of recipients and transferred non-surgically to the uterine horn ipsilateral to the position of CL. Ultrasonographic examinations were performed on Day 2 (to confirm ovulation); D9 (to measure corpus luteum size) and D30 (to determine pregnancy rate) for animals in both experiments. Data were analyzed by comparison of proportions between groups with GnRH and seminal plasma to ovulation rate, pregnancy rate and corpus luteum sizes.

Results and discussion

Experiment 1: Pregnancy rate in alpacas induced to ovulate with GnRH or Seminal plasma and inseminated

Results of Experiment 1 are shown in Table 1. Ovulation rates did not differ between treatments but the CL that formed after inducing ovulation with seminal plasma was significantly larger that the CL produced after GnRH injection ($p<0.05$). The pregnancy rate at D30 was also significantly higher ($p<0.05$) in alpacas induced to ovulate with seminal plasma compared with GnRH, but only if all the animals were included in the calculation. If just the animals that ovulated on D2 were included in the calculations there was no significant difference: 57.6 % vs 69.0 % for GnRH and Seminal plasma groups respectively.

Table 1: Pregnancy rates in alpaca artificially inseminated

Variable	**Induction of ovulation in alpacas artificially inseminated**	
	GnRH (n = 42)	**Seminal Plasma (n = 42)**
Follicle size (mm) Before treatment	8.5 ± 1.6	8.2 ± 1.4
Ovulation rate (%) (D2)	90.5 (38/42)	100.0 (42/42)
Corpus luteum size (mm) (D9)	10.6 ± 0.5^{a}	13.2 ± 0.7^{b}
Pregnancy rate (%) D30	$52.4\ (22/42)^{a}$	$69.0\ (29/42)^{b}$

[a,b] Values with different superscripts within the same row are significantly different $p<0.05$

Experiment 2: Pregnancy rate in alpacas induced to ovulate with GnRH or Seminal Plasma and used as embryo transfer recipients

The results of Experiment 2 are presented in Table 2. All the animals ovulated in response to either treatment and there were no significant differences between corpus luteum size (D9) and pregnancy rate (D30) between treatments.

Table 2: Pregnancy rates in alpaca embryo transfer recipients

Variable	Induction of ovulation in alpaca embryo recipients	
	GnRH (n = 11)	**Seminal Plasma (n = 13)**
Follicle size (mm) Before treatment	8.1 ± 1.8	8.3 ± 1.7
Ovulation rate (%) (D2)	100.0	100.0
Corpus luteum size (mm) (D9)	11.1 ± 0.4	13.8 ± 0.7
Pregnancy rate (%) D30	36.4 (4/11)	53.8 (7/13)

The results of Experiment 1 suggest that if seminal plasma is used to induce ovulation in alpacas that are inseminated, it has a positive effect on ovulation rate, CL size and pregnancy rate. These differences between treatments may be related to the significantly increased duration of the plasma LH surge, responsible for initiating ovulation that has been reported in alpacas treated with seminal plasma, as compared with those treated with GnRH (Adams et al., 2005). The larger CL produced in animals treated with seminal plasma has been reported to produce as much as double the progesterone secreted by the smaller GnRH induced CL (Adams et al., 2005), which could help improve pregnancy rate. The small number of animals in the second experiment may explain the absence of significant differences but both sets of results suggest that seminal plasma can be used as a non-hormonal alternative to increase the pregnancy rate when using assisted reproductive technologies in alpacas.

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Cryopreservation of epididymal sperm in alpacas

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Introduction

The use of assisted reproductive technologies for commercial alpaca farming in the United States has been slow to advance due to the lack of established semen cryopreservation methods. In particular, there is an essential need for the development of cryopreserving semen for valuable sires that are unable to reproduce due to relocation, illness, or death.

Earlier studies focused on cryopreservation techniques of ejaculated sperm in camelids and found problems associated with high viscosity and extremely low sperm concentration in the ejaculate (Garnica et al., 1993, Santiani et al., 2005).

Banda and coworkers (2010) described a technique for collection of epididymal sperm from alpacas in which sperm were collected after slaughtering or castration. Collection of epididymal sperm for cryopreservation avoids the viscosity and low concentration problems. In addition, Banda and colleagues (2010) compared cryopreservation of epididymal sperm using skim milk and Tris based extenders and found that viability and sperm membrane integrity were similar among the three extenders. However, skim milk based extenders yielded the highest post thaw motility.

In the United States, equine cryopreservation extenders are commercially available and readily used by large animal veterinarians for horses. Therefore, we hypothesized that these extenders could also be effective for cryopreservation of epididymal alpaca sperm. In addition, based upon the previous work of others, we hypothesized that skim milk based equine extenders consisting of glycerol would be superior to extenders without skim milk.

Methods

Four adult, intact male alpacas (mean ± SD: 7.5 ± 2.1 years) that were formerly herd sires and had recently sired crias were used in this study. Males were routinely castrated under general anesthesia (Telazol®, Wyeth, Parke, Davis & Company, Madison, NJ). Briefly, a scrotal incision was made over each testicle and the fascia surrounding the testicle was stripped. Then, the spermatic cord was crushed using a modified White's emasculator

such that a long section of vas deferens could be obtained (Figure 1). The skin incisions were allowed to heal by second intention. Immediately following castration, the vas deferens and cauda epididymides were dissected free from each testis (Figure 2).

Figure 1: Males were routinely castrated under general anesthesia and the spermatic cord was crushed using a modified White's emasculator to obtain a long section of vas deferens.

Figure 2: Vas deferens and cauda epididymides were dissected free from each testis for semen collection and cryopreservation.

For each side, the ductal lumen was slowly flushed in a retrograde direction with 1.0 mL of Tyrode's albumin lactate pyruvate and the sperm-containing effluent was collected into an Eppendorf tube. The sample was evenly aliquoted into three groups and extended at a 1:1 ratio with: Gent Extender for Frozen Stallion Semen (GE) (Minitube of America, Inc., Verona, WI), E-Z FREEZIN "Modified French" Equine Semen Extender (MF5) (Animal Reproduction Systems, Chino, CA), and E-Z FREEZIN "Lactose EDTA" Equine Semen Extender (LE) (Animal Reproduction Systems). The latter (LE) is not a skim milk based extender. Extended semen was loaded into labeled 0.5-mL cryopreservation straws, sealed, and frozen in liquid nitrogen vapor for 10 minutes before plunging into liquid nitrogen for storage. Semen was thawed at 38° C for 60 seconds and examined under phase microscopy at 400X magnification to determine total and progressive motility. In addition, semen was stained with eosin and nigrosin to determine post thaw viability.

Statistical Analysis

Mean±SD viability, total motility, and progressive motility were compared between extenders (GE, MF5, LE) using a one-way ANOVA. Significance was defined as $p < 0.05$.

Results

Dissecting and catheterizing the vas deferens and epididymides for flushing was not technically difficult. There were no significant differences in viability, total motility, or progressive motility between extender groups ($p = 0.60$, $p = 0.15$, $p = 0.12$, respectively; Table 1).

Table 1: Mean ± SD viability, total motility (TM), and progressive motility (PM) of cryopreserved epididymal alpaca semen after dilution in different commercial equine extenders. GE - Gent Extender for Frozen Stallion Semen, MF5 - E-Z FREEZIN "Modified French" Equine Semen Extender, and LE - E-Z FREEZIN "Lactose EDTA" Equine Semen Extender.

Extender	Viability (%)	TM (%)	PM (%)
GE	31.5 ± 21.8	0.5 ± 0.6	0.3 ± 0.5
MF5	56 ± 8.5	0.5 ± 0.7	0.5 ± 0.7
LE	45 ± 39.7	3.3 ± 2.9	3.3 ± 2.9

Discussion

A major consideration for determining success of alpaca semen cryopreservation protocols is the post thaw viability. For ejaculated alpaca sperm, Santiani and associates (2005) found that skim milk based extenders yielded greater post thaw viability (12.7 ± 5.9%) when compared to Tris based extenders. In the current study, the post thaw viability of epididymal sperm irrespective of extender was higher (41.4 ± 26.2%), but this was not significant due to the increased variation observed. Another major consideration for determining the success of alpaca semen cryopreservation protocols is the post thaw motility. For ejaculated alpaca sperm, Santiani and associates (2005) found that skim milk based extenders yielded greater post thaw total motility (15.3 ± 4.1%) when compared to Tris based extenders (4.0 ± 1.1%). With respect to epididymal alpaca sperm, Morton and colleagues (2007) found that lactose based extenders yielded greater post thaw total motility (18.2 ±

5.7%) when compared to Tris based extenders (11.3 ± 3.0%). In the current study, lactose based extenders yielded greater post thaw total and progressive motility when compared to skim milk based extenders, but this difference was not significant due to the small sample size. Additionally, our results may differ from earlier studies because of the type of sperm that was cryopreserved (ejaculated versus epididymal) and the type of extenders used (in-house extenders versus commercial equine extenders). Further studies comparing cryopreservation protocols are necessary before epididymal cryopreservation of alpaca semen can be applied commercially.

Conclusion

Based on the results of the current study, the data proved the first hypothesis that epididymal alpaca sperm could be cryopreserved using commercial equine extenders. However, the data disproved the second hypothesis in that skim milk based extenders did not yield higher post thaw motility.

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New developments on artificial insemination of llamas and alpacas

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Introduction

The use of artificial insemination (AI) has been instrumental in the genetic improvement of dairy cows and milk production has been improved tremendously by using semen from bulls with good dairy characteristics. The use of extended and then frozen/thawed semen has doubled milk production in countries where strict sanitary conditions are observed. In camelids the first cria born from AI was the result of a female alpaca having been inseminated with semen from a vicuna (wild camelid) more than 40 years ago. (Fernandez-Baca et al., 1968). One of the problems to overcome with AI in camelids is that the semen is very thick and gelatinous due to secretions from the bulbo-urethral glands. This seminal plasma holds spermatozoa in the uterus until the females ovulate after copulation with the male.

New method of semen collection

Semen collection from llamas and alpacas has been performed using different methods such as condoms, electroejaculation, urethral fistulation, artificial vagina (AV), deviation of the vas deferens and semen aspiration from the vaginal fundus. All these methods have advantages and disadvantages. The desire of owners to know the quality of semen ejaculated has led to an increased interest in collecting semen. Early on the examination of the bloody liquid emitted from the vulva of the female following copulation yielded the presence of spermatozoa. This observation and the gelatinous nature of the semen found near the external cervical os once the male has dismounted, led to the use of collecting semen by aspiration from the vaginal fundus. This method can be used under field conditions, as it does not need sophisticated materials or laboratory equipment.

Semen characteristics of semen collected by aspiration from the vaginal fundus compared with semen collected with an artificial vagina appear in Table 1.

Table 1: Semen characteristics from alpacas (n = 5 males) of ejaculated semen collected by vaginal aspiration (n=41), and by artificial vagina (n = 38)

Characteristics		Vaginal aspiration	Artificial vagina
Volume (mL)		3.6 ± 1.3[a]	1.5 ± 0.9[b]
Motility (%)		73.5 ± 7.9[a]	69.0 ± 14.4[b]
Spermatozoa concentration (10^6/mL)		75.20 ± 20.34[a]	80.34 ± 25.56[b]
Live spermatozoa (%)		75.3 ± 7.2[a]	70.8 ± 12.7[b]
Consistency (%)	Viscous	10	90
	Slightly viscous	90	10
Color (%)	Light red	80	0
	Dark red	10	0
	Milky white	5	60
	White	5	40

[a,b] Different superscripts between methods of semen collection ($p<0.05$)

Semen extension

There are many semen extenders used in llamas and alpacas; however, only a few have been used successfully, amongst which are Tris buffer mixed with hen's egg-yolk and lactose based extenders. However, there does seem to be a difference between alpaca and llama semen as, after dilution in tris-based extender with 20% egg-yolk and holding for 2h at 37 ºC, sperm motility was 73% in alpaca semen but only 45% in llama semen. Further work does need to be done to confirm these results. One of the improvements in semen extenders is the replacement of hen's egg-yolk with quail's egg-yolk. The fat globules are smaller in quails eggs compared with hen's egg-yolk so mixing semen with quail's egg-yolk is easier and faster than with hen's egg-yolk, and there is no need to use collagenase to eliminate the matrix of seminal plasma. The characteristics of semen extended with quail's egg-yolk are similar to that of any other extender and in the author's experience it does not harm the spermatozoa.

Artificial insemination

The first step in camelid AI is the induction of ovulation in the females to be inseminated. Two methods of inducing ovulation have been tested, firstly the administration of a hormone with luteinizing activity and secondly, breeding to a vasectomized male. In the first case, females are tested with a vasectomized male or with an intact male for sexual acceptance. Females that adopt the copulatory position are immediately injected with a

hormone with luteinizing activity, such as human chorionic gonadotropin (Chorulon®, Intervet, 750 IU, IM), or GnRH (Gonadorelin, 80 μg, IM). Results of inducing ovulation using these two hormones are illustrated in Table 2.

Table 2: Proportion of females ovulating following induction of ovulation with vasectomized males and gonadotropin releasing hormone (GnRH).

Species	**Vasectomized male**	**GnRH**	**Total**
Alpaca	57/79 (72%)	32/42 (76%)	40/49 (82%)
Llama	10/11 (91%)	6/6 (100%)	16/17 (94%)

The second method, induction of ovulation with a vasectomized male, has also been used in places where there is no budget to purchase hormones. The males, which had been vasectomized six months before doing any AI, mated the females 24 hours before AI. This resulted in more than 73% of females ovulating.

The effect on pregnancy rates of administering additional seminal plasma, previously collected from vasectomized males and sterilized by vapour pressure, after insemination has also been evaluated. Either, 0.5, 1.0 or 2.0 mL of sterile seminal plasma was inseminated into female alpacas immediately after they had been inseminated. Results of this study are presented in Table 3.

Table 3: Effect of seminal plasma on ovulation and pregnancy of alpacas inseminated with fresh extended semen.

Group	**Number of females**	**Females ovulating (Percentage of ovulation)**	**Pregnant females (Percentage of pregnancy)**
Control, no seminal plasma	19	15 (78.9)	8 (42.1)
0.5 mL seminal plasma	20	18 (90.0)	16 (80.0)
1.0 mL seminal plasma	18	13 (72.2)	8 (44.4)
2.0 mL seminal plasma	19	13 (71.5)	9 (47.4)
Total	76	59 (77.6)	33 (43.4)

The timing of AI is usually 24 to 26 hours after the induction of ovulation, however fixed-timed AI was also investigated. For this purpose, a group of 30 females were injected

with exogenous progesterone, (5 mg, i.m.) and after seven days all females had an ovulatory-sized follicle presents in their ovaries and were sexually receptive. They were therefore induced to ovulate with GnRH injection and inseminated 24 hours later.

Ejaculation is a continuous process during mating so when the male changes uterine horns he leaves some semen in the vicinity of the external cervical os. This semen can therefore by collected from the vaginal fundus close to the external cervical os immediately after the male has dismounted. The availability of this type of semen has eliminated the need for a phantom or AV. What is needed is a warmed vaginal speculum, a warmed 15 mL collecting tube and a male willing to breed for more than 20 minutes. Short breeding leaves less semen so it is more advantageous when the male breeds for more than 20 minutes. Once semen is collected it is evaluated under a microscope for motility before the extender is added. Semen is mixed with the extender and after about 10 minutes assessed for motility, concentration and viability. The concentration is adjusted to a final concentration 20 million spermatozoa/mL and then used immediately for insemination.

For insemination the female is restrained by three people, one holding the head and the other two holding the rear legs slightly raised. The vaginal area is cleaned with a wet towel and a vaginal speculum inserted. The external cervical os is localized and then a semen loaded insemination pipette is gently threaded into the uterine body where the semen is deposited. The next dose of semen is loaded into the insemination pipette by a second person and kept warm until the following female is ready for insemination.

The pregnancy rates of alpacas, kept under rural conditions, following insemination at 26, 28 and 36 hours after induction of ovulation are shown in Table 4.

Table 4: Pregnant rate of female alpacas at 30 days following artificial insemination at three different times after induction of ovulation.

Species	Induction of ovulation method	26 hours	28 hours	36 hours
Alpaca	Vasectomized	18/33 (54%)	12/24 (50%)	5/10 (50%)
	GnRH	4/16 (25%)	11/16 (69%)	13/16 (81%)
Alpaca	Vasectomized	8/8 (100%)	7/19 (37%)	5/29 (17%)
	GnRH	4/8 (50%)	1/18 (5%)	5/6 (83%)

This method of semen collection and insemination has been taken to isolated places, where alpacas live in the Peruvian Andes and where there is no laboratory equipment. The male

donating semen is chosen by the owner as well as the females. More recently, semen chilled for 12 to 24 hours has been inseminated and pregnancy rates of around 60 to 70% have been achieved. This has the added advantage that the male could be at a different location from the females.

In conclusion, artificial insemination of llamas and alpacas can be carried out under field conditions with limited laboratory equipment. Semen collection after copulation is an attractive alternative to using an AV especially when the owner wants crias out of their own males which may not be trained to a phantom and AV. Semen extension is possible with Tris-based extender plus 20% quail's egg-yolk (as an alternative to hen's egg-yolk) and the presence of blood components in the ejaculate is normal in llamas and alpacas due to the damage done to the uterine endometrium. The percentage of pregnant females after AI varies from 40 to 60% which is acceptable with a single insemination.

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Ovarian, uterine, and embryonic dynamics in early pregnancy in Alpacas

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Materials and Methods

Five females (4 - 13 years of age) were studied over the course of a year (Brunsden *et al*, 2012) all of which were proven breeders with at least one previous cria. The females were examined three days per week with at least one day between observations. At the start of each session, an intact male alpaca was brought to the group of females to determine their receptivity in a process known as behavior testing. After behavior testing the female, a transrectal ultrasound examination was performed using an Aloka SSD-500V, Portable Veterinary Ultrasound System with a 7.5 MHz linear transducer. If the female was receptive and had a significant follicle (≥5 mm in diameter) she was bred to one of the seven study males. The observations and ultrasound examinations continued on alternate days for 40 days and pregnancy was said to be established when the embryonic vesicle was visualized. If any follicles <5 mm in diameter were present, they were recorded as multiple small follicles and the diameter of the corpus luteum (CL) was also measured and its ultrasound appearance noted. The contractility of each uterine horn (seen as internal movement) was graded on a scale from 0 to 3 (none, low, medium, or high) and the diameter of each uterine horn was also recorded along with the amount of horn curvature. When the embryonic vesicle and embryo proper became apparent, their location and size was noted and the first appearance of the embryonic heartbeat on ultrasound examination was also recorded.

Results

Early embryonic death (EED) occurred in 4 of 10 pregnancies that were achieved during this study, with equal frequency for lactating and non-lactating females at the time of breeding. There was no difference in uterine contractility between pregnant and non-pregnant females, and no consistent pattern of contractility was seen with respect to establishment of pregnancy. Uterine contractility resulted in active deformation of the embryonic vesicle in all

pregnancies for the study animals, instead of maintenance of a spherical shape as in the horse. There was no significant difference in the diameter of the uterine horns noted in previously bred females before pregnancy. The embryonic vesicle was found consistently in one horn at approximately 20 days of gestation (estimated to be the time of implantation) before also being seen in the opposite horn. It was first noted in the left uterine horn on ultrasound examination in 7 of 9 pregnancies at least 3 days before it was also visualized in the right uterine horn.

Follicles of significant size (≥ 5 mm diameter) were found on the ovaries of all study females during all pregnancies. The largest was 12 mm in diameter, although the average diameter was 7 mm. On several occasions, a single ovary showed more than one significant follicle present and multiple small follicles (< 5 mm diameter) were also noted in some ovaries but not measured. Although a greater number of follicles developed on the ovary contralateral to the corpus luteum, follicles were also seen on the ovary ipsilateral to the corpus luteum. Some of these follicles were large in size thus proving that a CL and a large follicle can be present on the same ovary at one time. In contrast to ovarian follicular dynamics in non-pregnant females in one study by Donovan (2011), where non-pregnant animals did not demonstrate consistent follicular waves, waves of follicular development and regression were observed in the ovaries during pregnancy in the animals in this study. In 3 of the 4 pregnancies that resulted in EED, at least one significant follicle was found during almost every observation. In contrast, in 5 of the 6 pregnancies that were successfully carried past 40 days, follicles ≥ 5 mm in diameter were not often found. Additional animals would need to be studied before drawing any conclusion regarding the relationship of this finding to prediction of subsequent EED. The corpus luteum at the beginning of pregnancy was characterized by a bright white, echodense center and a white, echodense periphery on ultrasound examination. As the pregnancy progressed, the CL developed an echodense line across the middle and a less echodense peripheral outline. In the pregnancies with EED, the CL showed a uniform echotexture after early pregnancy. CLs were found on the left and right ovaries with equal frequency and 50% of the pregnancies originated from right ovary ovulations with the pregnancy established in the left uterine horn. Interestingly 75% of the pregnancies originating from right ovary ovulations also resulted in EED. Of the four pregnancies resulting in early embryonic death, three originated from the right ovary and one from the left. In contrast, three of the successful pregnancies came from left ovary ovulations and two from the right. In this study EED occurred more frequently when the ovulatory follicle was located on the right ovary, while successful pregnancies had a relatively equal

chance of originating from left or right ovary ovulations. The curvature of the uterine horns increased during pregnancy, most likely due to the influence of progesterone produced by the CL. Structural observations post breeding included: the corpus hemorrhagicum on Day 2, the corpus luteum on Day 4, the embryonic vesicle as early asDay 7, the embryo proper on Day 25, and the embryonic heartbeat on Day 27.

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Circulating progesterone concentrations and progesterone receptor expression in the camel uterus during early pregnancy

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Introduction

Circulating concentrations of progesterone (P4) during early pregnancy in mammals act on the uterine endometrium to establish uterine receptivity to implantation. Key to this is the continued exposure of the endometrium to P4 to induce down-regulation of the P4 receptor (PGR) from the luminal (LE) and superficial glandular (SG) epithelium; a process required to allow implantation in all mammalian species studied. However, to date no data are available on this phenomenon in camels. Luteolysis in camels occurs around day 8 post ovulation, so it is thought for this reason that changes in the uterine environment must occur at this time in order to maintain pregnancy. The aim of this study was to determine circulating concentrations of P4 in pregnant and non pregnant camels in which ovulation was induced. In addition we sought to determine the timing of loss of the PGR from the LE and SG of the endometrium in these animals.

Materials and methods

Animal model

Gonadotrophin releasing hormone (GnRH) was administered (Day -1) to induce ovulation (Day 0) in 43 female Dromedary camels with normal reproductive function. Approximately two thirds of these were inseminated (I; n=30) and one third were kept as non-inseminated controls (C; n=13). Blood samples were collected twice daily up until the day of endometrial biopsy on either Day 6, 8 or 12 post ovulation for I animals, and Days 6 and 8 for C animals. C camels were not measured up until Day 12 as camels have a luteal lifespan of only 8 to 10 days, and luteal regression occurs from days 9 to 12. This short luteal lifespan allows camels to return to a potentially fertile state more rapidly than other species. In the inseminated group the uterus was flushed with PBS before biopsy and animals were

further reassigned to pregnant (P) or non-pregnant (NP) groups based on whether or not an embryo was retrieved (P: n=15; NP: n=15).

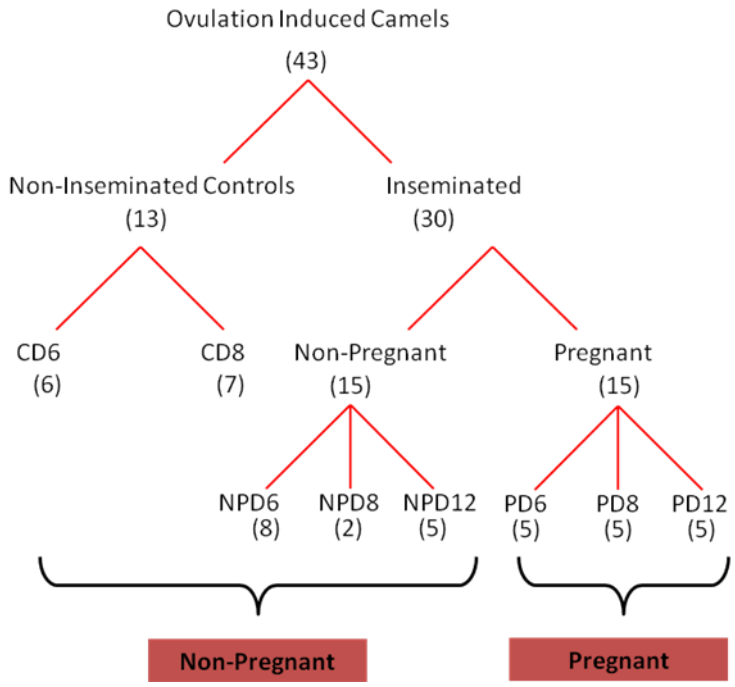


Figure 1: Experimental design. (C,control; NP, inseminated but non-pregnant; P, pregnant; D, day post ovulation). Numbers of animals are shown in parentheses.

Progesterone concentrations

The experimental design is shown in Figure 1. Serum P4 concentrations were measured using the Coat-a-Count® P4 kit (Siemens Medical Solutions. Diagnostics, Los Angeles, CA, USA). Differences among groups were determined using a repeated measures ANOVA in SAS with a significance set at $P<0.05$.

Quantitative Real Time PCR

Gene expression analysis for the *PGR* was carried out on a subset of the animals shown in Figure 1 (NP samples were not analysed; P, n=5 per treatment per time point; C, n= 6 per treatment per time point). Gene expression of the *PGR* in the endometrium on Days 6, 8 and 12 post ovulation of P, and Days 6 and 8 of C was determined using quantitative real time PCR (qRT-PCR). A dissociation curve was included to ensure the specificity of the amplification. A standard curve was included, as well as for the normalizer gene, *beta actin* (*ACTB*), to obtain primer efficiencies. Differences between groups were determined using an ANOVA with a significance set at $P<0.05$.

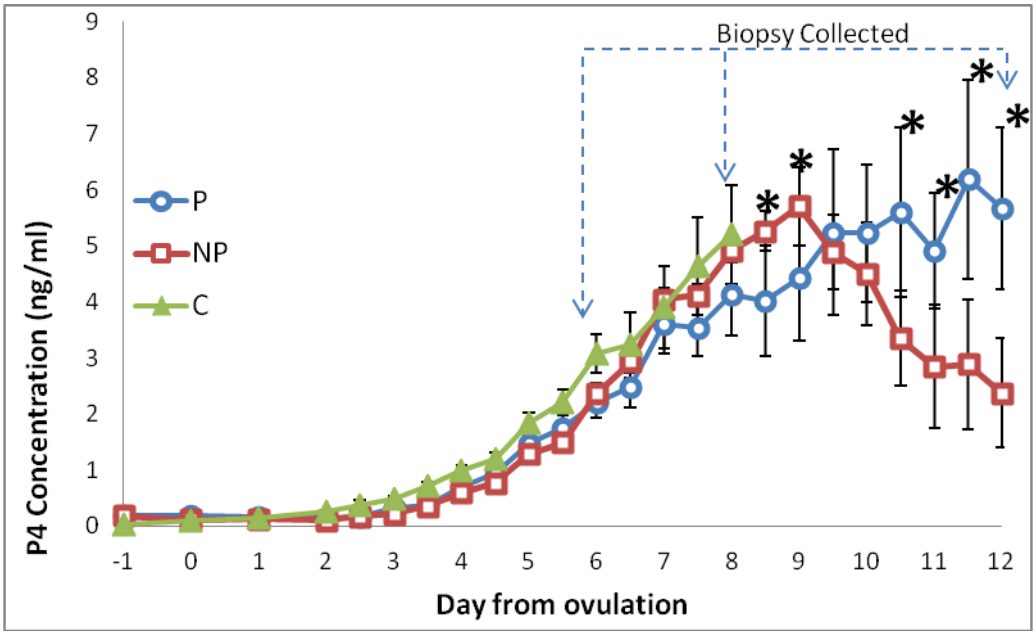
Immunohistochemistry

Localization of the P4 receptor isoform AB (PGR-AB) was carried out on paraffin embedded cross sections of endometrial biopsies taken from the endometria of P camels on Days 6, 8 and 12 and C camels on Days 6 and 8. The primary regions observed in the endometrium were the superficial glands (SG), the deep glands (DG), and the luminal epithelium (LE). Image Pro Plus software was used to measure the intensity of staining, returning an arbitrary value from 2 to 255. Background values (the intensity of negative controls containing no primary antibody) were subtracted from the intensity of test samples in order to obtain the final value. Differences between groups were determined using an ANOVA in SAS with a significance set at $P<0.05$.

Results

Circulating P4 concentrations (Figure 2) were not different among groups ($P=0.50$) but did differ over time ($P<0.0001$) and there was a group-by-time interaction ($P<0.0001$). Concentrations of P4 were similar between all three groups up to Day 8.0 ($P>0.05$). P4 concentrations were higher in NP than P on Days 8.5 to 9 ($P<0.05$) but lower on Days 10.5 to 12.0 ($P<0.05$).

Figure 2: Circulating concentrations of P4 in camels (n=43) from Day -1.0 (0= day of ovulation) to Day 8.0 in C, and Day 12.0 in P and NP (C, control; NP, inseminated but non-pregnant; P, pregnant; D, day post ovulation).

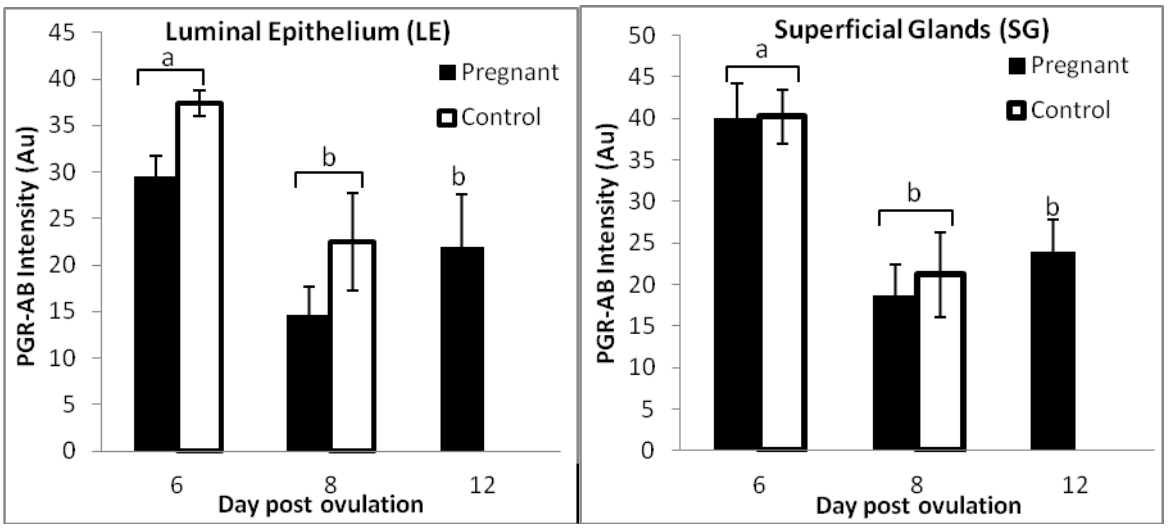


There was no effect of group, time, or the interaction for levels of mRNA for the *PGR* in endometrial tissues (P>0.05; data not shown).

PGR-AB localised to the LE, SG and DG of the endometrium on Days 6, 8 and 12 post ovulation in pregnant animals and on Days 6 and 8 for non-pregnant controls.

There was no effect of group, day or group-by-day for presence of PGR in the DG (data not shown). However, for the LE and SG there was no effect of group but there was a significant effect of day (Figure 3) with PGR intensity being greatest on D6 and lower on Days 8 and 12 (P<0.05). There was no significant group-by-day interaction.

Figure 3: Intensity of PGR-AB on Days 6, 8 and 12 in P and Days 6 and 8 in C camels in the LE and SG. Columns within panels with no common superscript indicate differences among Days (P<0.05)



Discussion

Loss of the PGR from the LE and SG of the uterine endometrium is required to establish uterine receptivity to pregnancy in mammals. Circulating P4 concentrations were higher (P<0.05) in P than NP camels after Day 10.5. Despite mRNA for *PGR* in homogenised uterine endometrium remaining constant on Days 6, 8, and 12, PGR protein abundance decreased in the specific compartments of the LE and SG in camels by Day 8.0 after ovulation. This change of protein localisation is consistent with observations in cattle and other ruminants. Elevated P4 induces down-regulation of PGR which in turn affects the expression of genes required for conceptus survival and growth, maternal recognition of pregnancy and implantation. It is proposed that this reduction of PGR is also required to establish uterine receptivity to pregnancy in camels.

Feasibility of ultrasonographic fetal gender determination in alpacas

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Introduction

Fetal gender determination can be used as a management tool by breeders. This tool allows prediction of the value of the pregnant female when cash flows depends on the gender of the fetus. Fetal gender can be determined ultrasonographically based on the location of the genital tubercle (GT). The GT initially develops between the hind legs on the ventral midline in both genders. As gestational age advances, the GT migrates towards its final location immediately caudal to the umbilical cord abdominal insertion in the male and ventral to the tail in the female. The GT becomes the penis in the male and the clitoris in the female. The GT is identified ultrasonographically as a hyperechoic structure, however, its image varies among species. This structure appears as an equal sign in cows (Ali, 2004) and mares (Curran and Ginther, 1989), and as a hyperechoic dot in ewes and does (Azevedo et al., 2009). Reliable fetal gender determination is only possible after migration of the GT is complete, so in most species, fetal gender determination is best performed between approximately 55 and 70 days of gestation. At the optimal time, diagnosis is possible on a single examination in > 80 % females with > 90 % accuracy (Ali, 2004; Azevedo et al., 2009; Curran and Ginther, 1989; Merkt et al., 1999).

To the authors' knowledge, no information has been published regarding the feasibility of fetal gender determination or the ultrasonographic characteristics of the GT in alpacas. The objectives of this study were to describe the ultrasonographic appearance of the GT and determine the feasibility of fetal gender determination in alpacas based on the location of the GT. Based on information available from other species, it was hypothesized that fetal gender determination based on the location of the GT would be accurate in alpacas when performed after 55 days of gestation, when migration of the GT is presumably complete, and until 70 days of gestation.

Materials and Methods

Adult pregnant huacaya alpacas (n = 10) with known mating dates were used in this study. Alpacas were between 46 and 64 days of gestation at the time of examination (Day 0 = last mating). One ultrasonographic examination was performed on each female for fetal gender determination. The females were restrained in a chute designed for alpacas. Initially, transrectal ultrasonography was done using an ultrasound machine (Aloka 900, Aloka Co., LTD, Wallingford, CT) equipped with a 5 MHz linear transducer in B-mode real time ultrasonography. The transducer was coupled with a rigid probe extension to facilitate manipulation without the need for insertion of the operator's hand and arm. Prior to ultrasonography, the rectum was instilled with 30 mL of obstetrical lubricant and the transducer was introduced into the rectum until an appropriate view of the fetus was obtained. If a diagnostic image was not obtained transrectally, the fetus was viewed transabdominally using a 3.5 MHz curvilinear transducer. Alcohol was applied to the skin of the left ventral and lateral abdominal wall and the transducer was applied cranial to the left inguinal area adjacent to the mammary gland and directed towards the opposite hip. This area contains thin and fine hair such that clipping was not needed.

Using either ultrasonographic view, the area from the umbilical cord to the fetal tail was examined to identify the GT. Fetuses were identified as male if the GT was immediately caudal to the abdominal attachment of the umbilical cord and female if the GT was located ventral to the tail. The predicted gender of the fetus and day of gestation were recorded at the time of examination, and the actual gender of the cria was recorded at birth. The diagnosis was considered correct when the predicted fetal gender agreed with the gender of the cria at birth.

Results

The GT in alpacas was a hyperechoic, elongated and bilobular structure having the appearance of an equal sign. Appropriate imaging of the fetus was possible in all attempts (10/10) using a transrectal or transabdominal approach. When fetal gender determination was performed between 59 and 64 d of gestation, all diagnoses were correct (6/6 = 100 %). However, when examinations were performed between 46 and 53 d of gestation, all diagnoses were incorrect (0/4 = 0 %) (Table 1).

Table 1: Predicted (ultrasound) and actual (at birth) gender of alpaca fetuses

Alpaca	Days of gestation	Predicted gender	Actual gender
1	46	male	female
2	46	female	male
3	53	female	male
4	53	male	female
5	59	female	female
6	59	male	male
7	60	male	male
8	61	female	female
9	63	male	male
10	64	female	female

Discussion

Fetal gender determination was accurate in alpacas between 59 and 64 days of gestation, but not prior to 53 days. Inaccuracy of early diagnosis possibly resulted from incomplete migration of the GT (Curran and Ginther, 1989; Merckt et al., 1999). Migration of the GT to its final location seemed to occur between 53 and 59 days of gestation. The most common reason for failure to reach a diagnosis with a transrectal approach was a fetus located deep in the abdomen and beyond the penetration of the transducer. As reported in other species, this made the fetal hindquarters inaccessible for imaging using the transrectal approach (Curran and Ginther, 1989; Merckt et al., 1999). Because a rigid extension was added, manipulation of the transducer within the rectum was limited and carried a risk for rectal perforation. Transabdominal ultrasound allowed visualization of fetuses positioned more cranially within the abdomen. However, manipulation of the large curvilinear transducer within the inguinal area was at times difficult. Flexion of the dam's left hindlimb decreased the space available for manipulation of the transducer. Use of the 5 MHz linear transducer for transabdominal imaging may accommodate easier manipulation within the inguinal area. The 5 MHz tranducer may not have sufficient penetration to view the fetus in some alpacas, but the higher frequency should yield higher resolution images and facilitate accurate assessment of the GT.

In summary, the ultrasonographic image of the GT in alpacas resembled that of bovine and equine fetuses. Fetal gender determination based on the location of the GT was

accurate between 59 and 64 d of gestation. At this stage, a diagnosis was possible in all alpacas when the transrectal and transabdominal approaches were combined. Further studies are needed to determine the true positive and negative predictive values of fetal gender determination in alpacas and the ideal time for diagnosis. However, this study provided an initial description and evidence of the feasibility of this technique in alpacas.

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Induction of abortion in alpacas using cloprostenol or dinoprost tromethamine

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Introduction

There are several situations in which it is desirable to terminate a pregnancy, yet retain an animal's ability to reproduce in the future. Examples would include a valuable breeding alpaca that is accidently bred by an "undesirable" male. The two most commonly used prostaglandins F2α (PGF2α) as a treatment for mismating in camelids are cloprostenol and dinoprost tromethamine. Cloprostenol is a synthetic PGF2α with a half-life of approximately 3 hours as it is more resistant to endogenous metabolism and is maintained in circulation for a longer time compared to dinoprost. Dinoprost is a natural PGF2α and its half- life is a few minutes (Martins et al, 2011). Interestingly, there is limited objective data to support either the safety or efficacy of these drugs for treating mismating in South American Domestic Camelids, essentially in alpacas. Severe hypertension and death following dinoprost administration has been reported in the llama (Smith et al, 2000), however no adverse reactions were observed following cloprostenol administration (Memon and Stevens, 1997). In the present study, we hypothesized that, in alpacas, cloprostenol or dinoprost treatment would induce abortion without severe complications while maintaining normal fertility in the treated females. The objectives of the study were to compare the efficacy of cloprostenol and dinoprost for induction of abortion in the first 5 months of pregnancy, to compare side effects of drugs and to compare the future fertility post treatment.

Material and Methods

Animal selection

Female alpacas (n=54) destined for meat production were selected for this study which was conducted at La Raya research center, in the region of Cusco, Peru; Latitude between 14^{o} 30’ and 15^{o} 45’ South; Longitude between 69^{o} 45’ and 75^{o} 00’ West; altitude

13779.5 ft above sea level. All animals were between 3 and 6 years of age, had given birth in the past year and were culled from the herd due to poor fiber quality. The study was conducted from June to July (winter season - 2011) and only females mated under an extended management system and between 2 to 5 months of gestation were included to the study. Animal selection criteria for inclusion in the study were, good general health, no prior reproductive disorders, and a body condition score between 3 and 3.5 (1= thin, 5 = obese). All females were examined by transrectal palpation and ultrasonography to determine pregnancy and were maintained under natural pasture management, supplemented with a mix of dry oats, hay and corn straw (500g per animal/day) with free access to fresh water.

Induction of abortion

Females were randomly distributed into two treatment groups; Group 1 (G1; n=26) received an intramuscular (i.m.) injection of cloprostenol (250 µg, Lutaprost® 250 Agrovet market) and Group 2 (G2, n=28) received dinoprost (5 mg, LutaLyse® - Pfizer) also i.m. All females were examined by transrectal ultrasonography every 24 hrs after treatment for abortion diagnosis. If a female failed to show signs of abortion the treatment was considered as a failure and further injections were administered. In those animals, a second dose was administrated 24 h after first injection, and if necessary a third and a fourth dose were administrated 48 hrs after the previous treatments respectively.

Breeding management

To evaluate fertility, follicular activity was monitored daily by transrectal ultrasonography using a 7.5 MHz linear transducer (Hitachi Aloka Medical, Ltd SSD model 500) from 15 days post-abortion in G1 and G2, and on a daily basis in G3 (control group) that were not pregnant, so had not received any treatment for induction of abortion. Then all females (G1, G2, and G3) were mated to a proven fertile male when the dominant follicle was between 7 to 12 mm in diameter and the uterus demonstrated maximum tone and oedema. Mating was considered adequate if it lasted a minimum of 15 minutes but was interrupted after 20 minutes and 50 µg GnRH (Cystorelin® US Merial) was administrated i.m. immediately after mating. To determine time of ovulation, mated females were examined by transrectal ultrasonography every 6 hours starting 24 hours after mating. After verification of ovulation in both treatment groups and the control group, females were randomly assigned to a group for embryo collection between 1 and 15 days post-ovulation (Day 0 = ovulation). For embryo collection, females were slaughtered according to approved methods set forth by

the Peruvian Veterinary Authorities. Immediately following slaughter, the reproductive tract was removed and transported to the laboratory where either the oviducts (of females from Day 1 to Day 6 post-ovulation) or the uterine horns (of females day 7 to day 15 post-ovulation) were flushed and the recovered medium examined for the presence of embryos to confirm pregnancy.

Results

In G1 abortion was induced in 7 /26 (26.9%), 16 /19 (84.2%), 2 /3 (66.7%), and 1 /1 (100%) females after the 1^{st}, 2^{nd}, 3^{rd} and 4^{th} dose respectively and in G2 it was induced in 3 /28 (10.7%), 18 /25 (72%), 5 /7 (71.4%), 2 /2 (100%) females after the 1^{st}, 2^{nd}, 3^{rd} and 4^{th} dose respectively. There were no significant differences between G1 and G2 treatments at the different doses (1^{st} dose P= 0.17; 2^{nd} P= 0.47; 3^{rd} P= 1; 4^{th} P= 1).

In G1 a total of 6 /26 (23%) actually expelled the fetus two days after the first treatment, whereas seven (27%) aborted three days after, five (19%) four days after, and two (8%) five days after the first treatment. The six (24%) remaining females aborted between 6 and 19 days after the first treatment. In G2: 1 /28 (3.6%) aborted two days after the first treatment, four (14.3%) aborted three days after, three (10.7%) four days after, six (21.4%) five days after, and five (17.9%) aborted six days after first treatment. The nine (28.6%) remaining females aborted between 7 and 18 days after the first treatment.

When comparing the side effects of drugs, 5 /26 (19.2%) females, and 12 /28 (42.9%) of the females presented complications from G1 and G2 group respectively but there was no statistical difference between treatments (P =0.082). None of these complications were severe, but the most common one observed, in both groups, was colic (discomfort, kicking the belly) approximately 12 h post treatment. In addition, a few females (5% for each group) had diarrhea 24 hours post treatment. At two to three months of gestational age, two females in G1 and three in G2 had a retained fetus which was then removed manually, however, none of them suffered from further complications or required any additional treatment.

Embryo collection rate was 65% (17 /26), 68% (19 / 28) and 83% (27 / 32) for G1, G2 and G3 (control group) respectively. There was no significant difference between groups (P = 0.1961).

Discussion

Cloprostenol and dinoprost have been recommended as luteolytic agents for use in llamas and alpacas (Smith et al, 2000). A case report of an elective abortion with cloprostenol in a llama (Memon and Stevens, 1997) showed that cloprostenol administered at the rate of

150 ug i.m. is an effective abortifacient. Cloprostenol appears to be safer and a more effective luteolytic agent than the natural PGF2α, dinoprost, as deaths and colicky reactions have been reported after administration of dinoprost (Smith et al, 2000). In the present study, a small number of females aborted after the first treatment in G1 (26.9%) and G2 (10.7%) but the majority of the females in G1 (84.2%) and G2 (72%) required a second treatment to induce abortion. The remaining females in both G1 and G2 aborted after a third or fourth treatment which was between 6 to 19 days post first treatment. Similarly, in a previous report that used cloprostenol in llamas, most of the abortions (83%) were induced after the second treatment (24 hr interval between 1st and 2nd treatment) which resulted in the fetus being expelled three to four days after the initial treatment, although some individual cases aborted as long as seven days after the first treatment (Smith et al, 2000). These results suggest that in order to induce abortion, it is necessary to use a second dose with an interval of 24 hr between treatments. Some animals are more resistant to a second dose, which may be due to individual physiological features or gestational age. In the current study 69% of abortions occurred two, three or four days following cloprostenol administration, and 64.3% aborted between 3 and 6 days following dinoprost treatment. There was no statistical difference between treatments, although this is probably due to the small sample size of each group. There were no deaths or serious complications in the females treated with dinoprost (Lutalyse), as reported in the previous study by Smith et al, (2000). Dinoprost has a very short half -life (minutes) compared to cloprostenol (3 hours), it is less resistant to endogenous metabolism probably due to its biochemical composition, and it is maintained in circulation for a shorter time than cloprostenol (Martins et al, 2011). These characteristics could explain the complications that result from dinoprost administration, as well as the prolonged time lag (days) between treatment and induction of abortion compared to cloprostenol. Concerning the side effects, although there was no statistical difference between treatments, the complication rate in females treated with dinoprost was higher (42.9%) than females treated with cloprostenol (19.2%). However, none of the complications needed additional treatment. In another study Bravo et al, (1996) compared the administration of fluprostenol sodium, dexamethasone, and oxytocin for induction of parturition in full term alpacas. Fluprostenol treatment was effective in inducing parturition and females delivered between 19 to 29 h following a single injection of the drug, while significant complications were associated with oxytocin and dexamethasone administration.

The embryo collection rate was compared between groups to evaluate fertility after induced abortion. There was no statistical differences between groups; G1 (65%) and G2

(68%) showed a very similar embryo collection rate, however, the control group of females which were not treated for induction of abortion showed a higher embryo collection rate (83%) compared to G1 and G2. Despite the fact that the females were evaluated for any reproductive abnormality before breeding and breeding was performed between 15 to 20 days post abortion, there is the possibility that some of the females needed a longer period of reproductive rest for at least three follicular wave cycles before being mated. Individual physiological features, age, body condition score, quality of the embryo, progesterone concentration, breeding season or male effect could also be some of the reasons to explain the lower collection rate in the treated females. Memon and Stevens (1997) reported an induced abortion of a 2.5 months old fetus in a mature llama following a single intramuscular injection of 150 µg cloprostenol, with fetal expulsion occurring approximately 108 h later. The animal was rebred 20 days post-abortion and carried the resultant cria to full term with normal delivery. In conclusion, the results demonstrated that pregnancy in alpacas can be safely and effectively terminated up to 5 months of gestation following cloprostenol or dinoprost administration without adverse effects on subsequent fertility. No statistical significance was observed between treatments; however our clinical observation allows us to recommend cloprostenol treatment.

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Investigations on clinical, immunological and biochemical aspects of Indian neonatal Dromedaries

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Introduction

The Indian single humped camel (*Camelus dromedarius*) exists under the agro-pastoral ecosystem of the Thar Desert. It plays a major role in the livestock economy of rural sectors of the arid parts of Rajasthan and Gujarat where special communities called "RAIKAS" and "REBBARIS" raise the camels. India stands tenth in the world for the largest camel population with 0.446 million camels out of the estimated 24.08 million worldwide (FAOSTAT, 2010).

Colostrum in the dam's milk is the most important nutritional factor for the newborn calf as it provides specific elements that enhance the calf's defense mechanism against disease and fulfills many nutritional needs. Neonatal care along with early recognition and treatment of clinical ailments are of utmost importance for reduction of neonatal camel calf mortality, thereby providing economic benefits to the camel owners. Neonatal pre-weaning mortality in dromedary herds raised under traditional systems was reported to vary from 10 to 30% (Arthur and Al-Rahim, 1982) whereas with improved management practices this can be reduced to below 5%. In India enteric/pulmonary infections were the most common causes of neonatal calf mortality in camels.

Materials and methods

A total of 6 pregnant healthy dams were selected each year and the following investigations were carried out every year over a span of 3 years. Immediately after calving (Day 0) and on Days 1, 3, 7, 15, 30, 45, 60, 75, 90 the body weights of the calves were recorded and average growth rate (AGR) calculated. In addition, on the same days blood samples were collected from healthy calves for serum separation and milk samples from dams for whey separation. The clinical aspects of temperature, pulse and respiratory rate

were recorded in the healthy calves 24 hours after birth and in clinically ill calves, below three months of age, when they showed clinical signs of digestive, respiratory, skin or certain other ailments. The immunological (serum and whey immunoglobulin G) parameters were estimated using single radial immunodiffusion test (Mancini et al., 1965). Certain biochemical estimations *viz.*, protein profile and glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine were estimated using standard estimation protocols in normal healthy calves, whereas the biochemical estimations (IgG, protein profile and glucose) in clinically ill calves were carried out at the farm prior to therapy. The data was analyzed statistically by utilizing SPSS 16.0 software.

Results and discussion

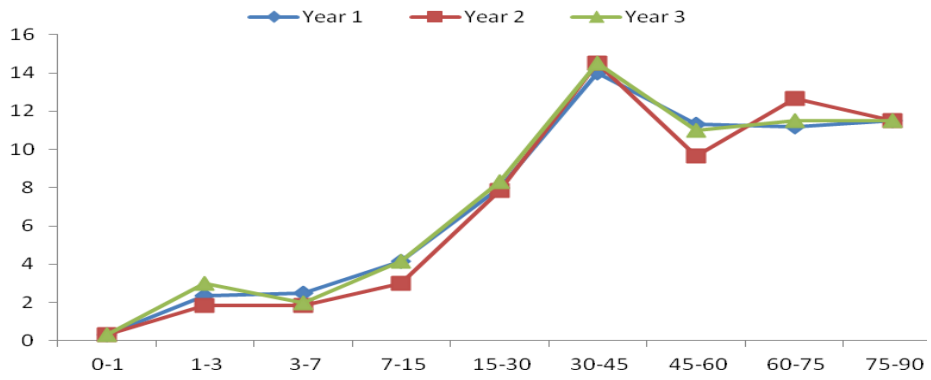
All the camel calves were born in a relatively advanced stage of development and held their heads up. The newborn camel calves weighed between 34 - 45 kg (mean 40 kg) and were up and walking within a few minutes to one hour after birth. In camel calves an epidermal membrane of fetal epidermal origin covering the neonates was seen at birth which was attached at muco-cutaneous junctions leaving nostrils or mouth uncovered; this was removed with the slightest friction. A few calves were seen to be shivering during the first few hours after birth but this is considered a normal phenomenon. The pooled mean data of three years (n=18) in healthy calves is presented below (Tables 1, 2, 3 and Fig. 1). The results of the mean body weight and average growth rate in calves of 0 - 3 months are shown in Table 1. and Figure 1. showing significance between days ($P<0.05$). The maximum increase in body weight was observed during the second month (25.26±0.81 kg) followed by the third (22.73±0.79 kg) whereas the first month was 16.60±0.62 kg.

Table 1: Mean body (±SEM) weight and average growth rate in neonatal camel calves (0-3 months)

Days	B. wt.* (Kg)	AGR* (Kg)
Zero	40.38±0.82	-
1	40.67±0.79	0.27±0.15
3	43.05±2.95	2.66±0.21
7	45.16±0.69	4.77±0.39
15	48.94±0.53	8.55±0.47
30	57.00±1.81	16.61±2.54
45	71.33±0.96	30.94±1.22
60	82.00±0.51	41.61±1.02

*Between days ($P<0.05$)

Figure 1: Mean average growth rate



The pooled mean (±SEM) of serum and whey immunoglobulin G levels are shown in Table 2. The new born camel calves were a/hypogammaglobulinaemic but within 2-3 hours the calves were made to suckle their dam for colostrum. All the camel calves then showed >10 mg/ml of Ig G by 72 hours indicating successful passive transfer (Wernery, 2001). In other livestock species failure of passive transfer (FPT) of immunoglobulins was considered critical for the health and survival of the calf (Cabell, 2002) and successful immunoglobulin transfer was reported to be associated with low infection rates and high likelihood of survival (McGuire *et al.*, 1976). Neonatal calves have been born agammaglobulanaemic in cattle (Logan *et al.*, 1974), buffalo (Lone *et al.*, 2003), and llamas (Bravo *et al.*, 1997) but the Ig levels increase once they started taking colostrum.

Table 2: Mean (±SEM) Immunolgobulin levels (mg/ml)

Days	Serum Ig's*	Whey Ig's*
Zero	0.03±0.006	18.90±0.27
1	5.02±0.01	17.22±0.18
3	10.15±0.32	11.92±0.32
7	10.90±0.08	10.65±0.23
15	11.40±0.07	8.21±0.27
30	11.72±0.05	7.25±0.09
45	11.95±0.02	6.06±0.06
60	12.63±0.07	5.33±0.39
75	13.58±0.08	4.19±0.13
90	14.03±0.03	3.28±0.10

*Between days ($P<0.05$)

Over a span of 3 years in healthy camel calves the pooled mean (±SEM) of serum biochemical parameters *viz.*, serum glucose and total protein, albumin, globulins and A/G ratio on different days are depicted in Table 3. In healthy calves of 0-3 months of age the ALT levels ranged between 4 - 12 U/L, AST levels within 95 - 122 U/L, urea concentration varied from 21-42 mg/dl and creatinine levels from 0.4 - 0.9 mg/dl. Significant variations in the biochemical profile were noticed on different days until one month of age. In normal healthy calves the temperature varied from 37.5 to 38.5^{o}C, pulse rate from 90 to 110 beats per minute and respiratory rate from 24 - 28 breaths per minute.

Table 3: Pooled Mean (±SEM) serum biochemical estimations in neonatal camel calves (0-3 months)

Days	Glucose* (mg/dl)	Total protein* (g/dl)	Albumin*(g/dl)	Globulin*(g/dl)	A/G ratio*
Zero	32.24±0.79	4.99±0.03	3.12±0.008	1.86±0.03	1.68±0.03
1	44.11±0.47	5.21±0.03	3.11±0.01	2.10±0.04	1.49±0.03
3	47.68±0.56	5.65±0.06	2.98±0.03	2.66±0.08	1.14±0.04
7	49.45±0.62	5.97±0.07	3.01±0.02	2.95±0.06	1.02±0.01
15	51.37±0.49	6.32±0.04	3.12±0.01	3.20±0.04	0.97±0.01
30	56.32±0.52	6.56±0.03	3.21±0.01	3.34±0.03	0.96±0.01
45	56.64±0.60	6.46±0.03	3.21±0.008	3.25±0.03	0.98±0.009
60	57.54±0.36	6.58±0.02	3.26±0.01	3.31±0.02	0.98±0.009
75	57.68±0.41	6.57±0.03	3.27±0.01	3.30±0.03	0.99±0.011
90	59.90±0.48	6.64±0.03	3.31±0.01	3.33±0.02	0.99±0.04

*Between days ($P<0.05$)

Commonly noticed infections in camel calves below 3 months of age were enteritis, pneumonia, heat stress, ectoparasites (ticks, mange mites) and endoparasitic infections. In pneumonia there was an increase in body temperature (39 - 40.5^{o}C) and pulse rate (98 - 116 per minute) with dyspnoea. In ecto and endoparasitic infections the body temperature varied from 38.0 to 38.5^{o}C with a slightly lower pulse rate compared with values at 24 hours after birth; which may be physiological with increase in age. In enteritis a slight increase in temperature was noticed in a few cases whereas others were within the normal range, but the enteritic calves that died revealed a subnormal temperature prior to death. Changes in the glucose and protein profile and immunoglobulin levels were recorded in camel calves that

showing clinical signs of enteritis, ectoparasitic, endoparasitic infections and pneumonia. For example hypoglycemia (46-54 mg/dl), hypoproteinemia (4.84 to 5. 08 g/dl) and decreased Ig levels (10.48 to 11.26 mg/ml) were noticed when compared with normal healthy calves. In extreme winters cases of pneumonia were encountered, whereas in summer mortality due to heat stress was more common. Earlier analysis of data on causes of mortality in camel calves also showed that enteritis and pneumonia were the major causes followed by heat stress (Ali *et al.*, 2006; Sena *et al.*, 2006).

Acknowledgements

I am highly thankful for the Director and Staff of Livestock Farming Unit of NRC on Camel, for providing the facilities to undertake the work.

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Embryo transfer in alpacas

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The development of artificial breeding technologies in alpacas will increase the use and allow more economic movement of genetically superior animals nationally and internationally. Generation intervals are relatively long in alpacas because males are slow to sexually mature and females exhibit an extended gestation (11.5 months), so conventional breeding results in slow genetic gain. Assisted breeding technologies are being used to improve wool quality more rapidly than would otherwise be possible by natural mating in industries such as Merino sheep and Angora goats. However, the reproductive physiology of alpacas differs to that of other domestic livestock and remains poorly understood, therefore hindering the direct transfer of artificial insemination (AI) and embryo transfer (ET) technologies from ruminants to alpacas.

The understanding of ovarian function in alpacas has been instrumental in the success of developing non-surgical, transcervical single and multiple ovulation ET. Females exhibit waves of ovarian follicular growth, with new waves emerging every 12 to 22 days (Vaughan et al., 2004). Females are induced ovulators, and ovulate 30 hours after copulation when they have a dominant follicle of at least 6 mm on either ovary (Adams et al., 2001; Bravo et al., 1991). A corpus luteum develops on the ovary at the site of ovulation 3-4 days after mating and secretes progesterone. If conception does not occur, prostaglandin is released from the uterus and induces regression of the corpus luteum 10-12 days after mating (Adams et al., 1989). The embryonic signal for maternal recognition of pregnancy must be transmitted as early as Day 9 or 10 after mating in order to 'rescue' the corpus luteum of pregnancy, as the corpus luteum is the major source of progesterone throughout pregnancy.

Single ovulation vs multiple ovulation

Single ovulation embryo transfer of alpacas does not require any hormonal treatment of donor females (Taylor et al., 2000). Donor females are mated once and flushed a week later. Approximately 7 of every 10 females flushed will produce an embryo (Vaughan et al., 2012). Follicle growth in the first 10 days after new wave emergence is consistent regardless

of subsequent interwave interval (Vaughan et al., 2004), an observation integral to the success of single-embryo flushing of donor females every 10-12 days. More than 400 live births (50 % males, 50 % females) have occurred over the last 8 years in Australia, following single-embryo flushing in numerous commercial alpaca herds (Vaughan et al., 2012). Donor females have since given birth to crias from matings performed soon after embryo flushing, indicating donor fertility was not interfered with during embryo collection.

Methods of multiple ovulation and embryo transfer (MOET or 'superovulation') are also being implemented in alpacas in Australia and other countries. Both equine chorionic gonadotrophin and follicle stimulating hormone are currently being used as agents to stimulate multiple ovulation. Techniques are producing an average of 2.5-3 embryos per flush (up to 21 embryos per individual) on most farms (Ratto et al., 2012; Vaughan et al., 2012). Results have been less reliable on some farms, presumably due to variations in alpaca fertility, nutrition, environment and management. The number of studies on MOET in camelids remains low and further refinement of existing protocols is continuing to identify a MOET program that consistently yields an acceptable number of transferable embryos, and is associated with minimal risk of infertility to the elite donor female. Embryos have been yielded on many consecutive MOET programs in the last eight years in Australia, without apparent effect on donor fertility as donor females have readily conceived within 2-4 weeks after their last MOET flush.

Preparation of donors and recipients

Females that are to be used as donors need to be reproductively sound (owners must resist the temptation of preparing females that have been difficult to get pregnant in the past), of superior genetic quality, have good conformation, and be free of all known inherited genetic disorders.

Females that are to be used as embryo recipients must also be reproductively sound in order to optimise the chances of successful embryo implantation and birth of a cria. Demonstrated good mothering ability is an advantage. Females with physical and/or genetic abnormalities (carpal valgus, luxating patellae, fused toes, extra toes, wry face) can be used as recipients since these characteristics will not be transferred to the embryo and gestating foetus.

Attention to detail and thorough preparation of donor and recipient females (and males) is essential for successful embryo transfer. Three factors appear to be important for all

alpacas participating in an ET program: 1. normal fertility, 2. body condition score 2.5 to 3 (out of 5), 3. adequacy of selenium intake.

Most females ovulate one egg after mating, with multiple ovulations occurring in up to 10 % of natural matings. Ideally, donor females should have their ovaries examined by ultrasonography every second day to monitor follicular wave patterns. When a growing or mature follicle (greater than 6 mm diameter) is present on an ovary, the donor female can be mated to a genetically superior male. If adequate numbers of recipient females are available they should be subjected to ovarian ultrasonography every second day in order to monitor follicle growth. Females with a growing or mature pre-ovulatory follicle on the day the donor female is inseminated should be induced to ovulate.

Both donor and recipient females are induced to ovulate at a similar time so that the uterine environment and circulating progesterone are comparable for donors and recipients. Hormones that can be used to induce ovulation include GnRH analogues, such as buserelin, and after injection of GnRH, around 90 % of females ovulate within 30 hours.

Embryo development in camelids

The embryos of camelids develop faster than in domestic ruminants and morulae have been recovered in the oviducts of llamas as early as 3 days after mating. The faster rate of embryo development in camelids is likely to be related to early maternal recognition of pregnancy, which needs to occur around Day 8 to 10 after mating to ensure persistence of the corpus luteum of pregnancy (Aba et al., 1997).

Embryos are flushed from donor females 7-8 days after mating and are usually in the form of a hatched blastocyst. Embryos are usually spherical and up to 4 mm in diameter at this time. Elongation of blastocysts occurs from Day 7 or Day 8 after ovulation and by Day 14, blastocysts can be 10 cm in length.

Non-surgical, trans-cervical collection and transfer of embryos

This method involves the introduction of a Foley catheter through the cervix and placement of the catheter in the uterus (Vaughan et al., 2012). Medium is flushed through the catheter into the uterus, then allowed to drain, via gravity, into an embryo collection vessel. This method is relatively non-invasive and does not have the attendant risks of abdominal adhesions associated with surgical embryo collection. However, females with a narrow pelvis or excessive fat in their pelvis may not be suitable for non-surgical collection and there is also a risk of rectal trauma with this procedure.

The retrieved fluid is examined under a dissecting microscope for embryos. After collection and washing, single embryos are loaded into small plastic straws, similar to those used for artificial insemination, and then placed transcervically (non-surgically) into the uterus of the recipient female. Pregnancy diagnosis using transrectal ultrasonography can be performed from approximately Day 25 after embryo transfer to assess pregnancy (Parraguez et al. 1997) and approximately 50 % of transferred embryos reach full term.

Future developments in embryo transfer in alpacas include the continued refinement of multiple ovulation protocols and the freezing of embryos to allow indefinite storage and easy transport of genetic material. Pregnancies have been achieved in camels (Skidmore et al., 2004) and llamas (Aller et al., 2002; Skidmore et al., 2004; Taylor et al., 2005) following vitrification or slow freezing, thawing and transfer of embryos, but this success has not yet been translated to alpacas.

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Ovarian superovulation in South American camelids

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An increasing interest in the production of South American camelids has developed over the last few years. Camelids possess unique reproductive characteristics that constitute a challenge for the development of assisted reproductive techniques. Gestation length in these species varies between 335 and 360 days and only one offspring is born per year. Therefore, the average number of progeny any female can produce throughout her reproductive life is limited, making it hard to effectively spread a desired genome. Applying reproductive biotechnologies offers the possibility of increasing genetic progress and reproductive efficiency with the aim of optimizing reproductive management of genetically superior females.

Control of ovarian follicular growth: follicle wave inhibition

The need to produce multiple dominant follicles from one follicular wave requires the use of superovulatory treatments. According to Bourke et al. (1995a), when implementing ovarian superstimulation it is necessary to start hormone treatment in the absence of dominant follicles. Moreover, Miragaya et al. (2006) observed that when starting the treatment in the presence of a follicle larger than 5 mm, growth of only that follicle is induced. Due to these findings, superstimulatory treatments are applied to donor females in the absence of follicles greater than 5 mm (controlled by ultrasound).

Treatments applied

To achieve absence of dominant follicles prior to applying a superstimulatory treatment, some researchers have carried out the manual rupture of follicles greater than 5 mm by transrectal manipulation (Sansinena et al., 2007) or ablation of the follicle using transvaginal ultrasound-guided follicular aspiration (Ratto et al., 2003; 2005). Others have applied a superstimulatory treatment when the absence of follicles greater than 5 mm was confirmedby ultrasonography (alpacas: Ratto et al., 2007; llamas: Bravo et al., 1995; Berland et al., 2011; vicunas: Chaves et al., 2004). In addition, various protocols have been developed

to inhibit ovarian dynamics based on the negative effect progesterone has on follicle activity in the presence of a corpus luteum (Aba et al., 1995). In this respect, a natural luteal phase, produced by inducing ovulation of the dominant follicle (Bourke et al., 1992, 1995b) or an artificial phase, by applying progesterone or exogenous progestogens, can be used. The latter can be found as injections (short or long-acting progestogens) or as releasing devices: subcutaneous implants with progesterone (Norgestomet®, 3 mg; Bourke et al., 1992, 1995b), intravaginal devices (CIDR®, 0.33 g; alpacas: Velásquez and Novoa, 1999; llamas: Bourke et al., 1992; Chaves et al., 1998, 2002; Cue-mate®, 0.78 g, Cavilla et al., 2006) or intravaginal sponges with medroxiprogesterone (MAP) (alpacas: Gamarra et al., 2006; llamas: Huanca et al., 2009). Progestogens can also be combined with injectable estrogens (17 β estradiol, estradiol benzoate, estradiol valerate). Alberio and Aller (1996) used 50 mg of injectable progesterone over a period of 12 days, obtaining on Day 7 the inhibition of follicle diameter below 5 mm. Carretero et al. (2010) evaluated the efficacy of administering 100 and 150 mg of progesterone daily for 5 days, and observed that follicles decreased their size to 5 mm as early as day 3. This protocol is highly effective, the disadvantage being that it is not practical for use in the field (daily injections are needed) and it produces pain at the site of injection. For these reasons it would be useful to evaluate the effect of long-acting progesterone (requiring only one injection; BioRelease™ LA 300, BETPharm) on ovarian dynamics in the llama. Its formulation has been prepared to release progesterone for approximately 10 to 12 days after its i.m. administration. In horses it has been proven that 1500 mg of this compound maintains high progesterone levels (> 4 ng/ml) for 10 days (Burns et al., 2008). Our group has started to evaluate its effect in llamas and our preliminary results show a decrease in the size of the dominant follicle in 75% of the females 6 days after a single dose of 300 mg of progesterone. Initial plasma progesterone levels measured by radioimmunoassay showed a pronounced increase followed by a gradual decrease in concentration. According to these results, progesterone levels declined to < 2 nmol/l seven days after injection of progesterone BioRelease™ LA 300, therefore day 7 post-injection would be the best day to start ovarian superstimulation (Trasorras et al., unpublished data).

Ovarian superstimulation

The hormones most used to induce ovarian superstimulation in camelids are FSH and eCG, either individually or combined. However eCG is effective with a single administration, therefore with regard to reproductive management, it is more convenient to use than FSH,

which has a shorter half-life and therefore needs repeated doses over a period of time (Bravo et al., 1995; Agüero et al., 2001).

According to Bravo et al. (1995) administration of 500 or 1000 IU of eCG, in the absence of dominant follicles (controlled by ultrasound), are the optimal doses for superovulation in llamas and an increase in the incidence of cystic follicles was observed with higher doses (2000 IU). Trasorras et al. (2009) reported that 500 IU of eCG did not produce ovarian superstimulation after follicle inhibition with estradiol benzoate and CIDR®. In alpacas, administration of 1000 IU of eCG at the end of a CIDR® protocol produced a higher ovarian response (number of corpora lutea) than treatment during the follicular phase (no progesterone treatment); besides, females treated with eCG without pre-treatment with progesterone had a significantly higher incidence of follicular cysts (Velásquez and Novoa, 1999). According to our experience, treatments with 1000 or 1500 IU of eCG are effective for inducing multiple follicle growth in llamas, but administration of 1500 IU produces a higher number of follicles (Trasorras et al., 2009; Carretero et al., 2010). Administration of high doses of eCG can be beneficial for carrying out follicle aspiration to obtain oocytes for use in assisted reproductive techniques such as IVF and ICSI. On the other hand, when the objective is to obtain embryos through uterine flushing, its use could be detrimental due to the possible displacement of the ovarian bursa because of the large size of the superstimulated ovary. In vicuna, administration of a dose of 750 IU of eCG produced a potent stimulation of follicle growth, obtaining an average of 16.5 follicles per female (Aba et al., 2005). In these cases displacement of the ovarian bursa was observed and no embryos or oocytes were obtained from the uterine flushings, with the apparent loss of oocytes into the abdominal cavity (Agüero, personal communication).

In the near future, advances in technology will make new species specific recombinant hormones available for use in these species, thus opening new possibilities for obtaining better results with ovarian superstimulation.

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Effect of holding temperature on pregnancy rate of Llama embryos

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Introduction

Llama (*Lama glama* × *Vicugna pacos*) herds are typically spread at remote distances in Argentina so a protocol permitting embryo holding for several hours would facilitate embryo transfer when donor and recipient females are located at remote distances.

Previous studies in llamas, demonstrated a pregnancy rate of 33 and 50% for fresh (n = 6) and vitrified embryos (n = 4), respectively (Aller, 2002) and Sumar (2011) reported a pregnancy rate of 53% after transfer of fresh alpaca embryos. In camels, McKinnon et al. (1994) and Skidmore et al. (2002) have reported pregnancy rates of between 50 and 70% when Day 7 embryos are transferred into synchronized recipients.

When transferring from one herd to another, holding fresh or chilled embryos for extended periods of time might be an alternative to embryo vitrification. The objective of this study was to determine pregnancy rate after an 18-hour holding period of llama embryos at either 4 or 25°C before transferring to recipients.

Materials and Methods

This trial was performed in a commercial farm located at Saavedra (Buenos Aires, Argentina) during the spring and summer season (October 2011 through March 2012). The mean annual rainfall is 600 mm concentrated in spring and summer. The animals were kept in natural pasture and water was provided ad libitum.

Superovulatory treatment and embryo transfer

Donor females were injected intramuscularly (i.m.) on days -7, -5 and -1 relative to mating (i.e., day 0) with 4.2 μg of GnRH (Receptal®, Hoechst), 1000 IU of eCG (Novormon®, Syntex, Argentina), 250 μg of $PGF_{2\alpha}$ (Estrumate®, Schering-Plough Animal Health), respectively. At day 0, donor females were injected with 4.2 μg of GnRH (Receptal®, Hoechst) i.m and naturally mated, then embryo collection was performed non-

surgically 7.5 days after mating. Collected embryos were maintained according to one of the following treatments: EquiPro® holding media (Minitube, USA) within an Equitainer (Hamilton Research, Inc.) system at 4°C (n = 20) or Syngro® holding media (Bioniche Animal Health, Inc.) within a temperature controlled semen storage unit (Minitüb, Germany) set at 25°C (n = 22). All embryos were transferred into recipient females 18 hours ± 75 minutes after embryo collection. Recipient females were injected with 4.2 μg of GnRH (i.m.) 12 hours after donors were mated, and with 50 mg of hydroxyprogesterone caproate (Proluton® Depot, Bayer, Argentina) 24 hours before embryo transfer. Pregnancy was determined at 21 and 45 days by transrectal palpation and ultrasonography using a 5-MHz probe.

Embryo recovery

Embryo collection from the donors was performed non-surgically on day 7.5 after natural mating. Each llama was restrained in a special llama chute and sedated with a single injection of 1.8 mg of acepromazine (i.m.). Each uterine horn was flushed using a 16 Fr Foley catheter and 250 ml of Lactated Ringer's and the flushing solution was passed through an EmCon® filter (Minitube, USA). The recovered media remaining in the filter was examined under a stereomicroscope (Olympus SZ51 zoom stereo microscope) and all located embryos were washed three times in fresh media and then transferred to an embryo holding tube with the appropriate holding medium (EquiPro® vs. Syngro® Holding).

Statistical Analyses

Statistical analysis was performed as a two-proportion test using Minitab 15 Statistical Software (Minitab Inc., 2007). Statistical difference and tendency to difference were declared at $P < 0.10$ and $P < 0.15$, respectively.

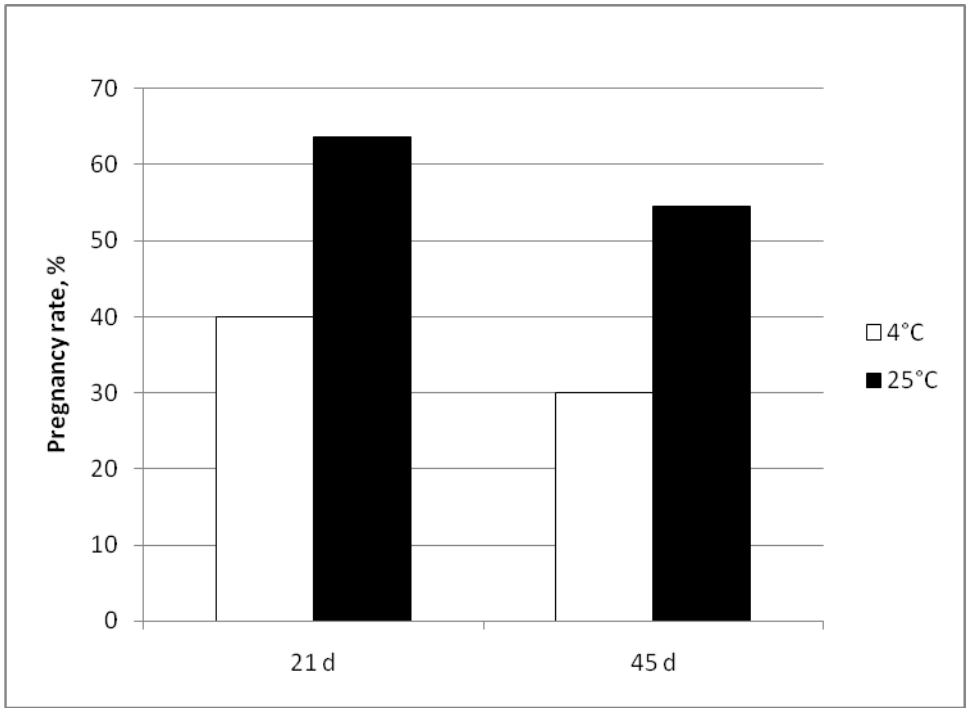
Results and Discussion

Pregnancy rate tended to be greater ($P < 0.12$) for embryos maintained at 25°C than for embryos maintained at 4°C (63.6 and 40.0% pregnancy rate, respectively) when pregnancy was confirmed after 21 days. When pregnancy was confirmed at 45 days, pregnancy rate was also greater ($P < 0.10$) for embryos maintained at 25°C than for embryos maintained at 4°C (54.5 and 30.0% pregnancy rate, respectively). The differences in pregnancy rates between treatments at the different pregnancy confirmations do not have a biological explanation. Analyzing the reduction in pregnancy rate within treatments at 21 and

45 days found that they were not different (P < 0.55) among treatments (14.3 and 25% reduction for embryos maintained at 4 and 25°C, respectively).

Holding fresh embryos for an 18-hour period at 25°C allows transferring embryos from one herd to another when kept at remote distances from each other, even though from a logistic perspective, embryo vitrification may seem to be a more practical alternative for this purpose. Even though Aller et al. (2002) reported 50% pregnancy rate for vitrified embryos in llamas, this data was obtained with only four transferred embryos, so further work needs to be done to verify those results. Whether pregnancy rate in a large number of animals is similar between embryo vitrification and embryo holding for an 18-hour at 25°C still needs to be elucidated.

Figure 1: Pregnancy rate in llamas after 21 and 45 d.



Conclusion

In conclusion, maintaining embryos for 18 hours at a controlled temperature of 25°C seems to be an adequate protocol to permit embryo transfer when donor and recipient females are located at remote distances.

Acknowledgements

I want to thank Gonzalo Ferreira (PhD) for performing all statistical analyses of this research. Also, I want to thank everybody at Estancias y Cabaña Las Lilas and, especially to Don Miguel Castiglioni for all the help and support during this study at La Josefina.

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Reciprocal embryo transfer in alpacas and llamas

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Introduction

The intraspecific transfer of mammalian embryos has now been developed into a well-established technique (Betteridge, 1977) and several research reports in South American Camelids (SAC) reveal many significant benefits of using intraspecific embryo transfer (ET). For example, it permits a rapid repopulation of camelids, it enables the preservation of endangered species and breeds and more offspring can be produced from donors with high genetic merit (Bourke et al., 1991; Del Campo et al., 1995; Correa et al., 1992; Vaughan and Tibary, 2006; Huanca et al., 2007; Sumar, 2008). However, relatively little information is available concerning interspecific transfer in domestic animals, in which the conceptus and female carrying the pregnancy are of different species. From a practical perspective, interspecific ET is viewed as a means of preserving endangered mammals (Anderson, 1988), but for this to be successful it requires effective placentation which relies upon glycocompatibility or the presence of specific "glycotypes" (Jones et al., 1997). In SAC a few interspecific ET reports have been published with promising results (Taylor et al., 2001; von Baer et al., 2003; Sumar, 2008). Therefore the aims of this study were to: 1) determine whether alpaca embryos could develop normally in llama recipients and whether they would have higher birth weight and better body development during the first few months of life; 2) determine if llama embryos could develop normally in alpaca recipients; 3) investigate the so called "genetic distance" between these two species and 4) look at the glycocompatibility of both species at the fetomaternal interface.

Material and Methods

This experiment was done at the SUMAC TARPUY Laboratory at Ayaviri, Puno, located in the South Andean altiplano of Peru, at an altitude of 3,950 meters above sea level. Alpacas and llamas were kept on natural pastures, supplemented with alfalfa and oat hay. The animals were assigned to the following groups: Group 1 (G1) consisted of four alpaca donors and 10 female llamas recipients; Group 2 (G2) consisted of two llama donors and

three alpaca recipients. In both groups donors and recipients were examined daily by transrectal ultrasonography (ALOKA 500D, with a 7.5 MHz Transducer, Japan) and matched according to follicular maturation. Donor females were mated with fertile males of the same species when a mature follicle ≥ 7.0 (alpacas) or 9.0 mm (llamas) was detected in their ovaries, and each female was injected with 1.0 (alpacas) or 1.5 ml (llamas) GnRH (0.0042 mg of buserelin acetate / ml, Conceptal®. Intervet; i.m) immediately after mating to aid ovulation. For synchronization, the recipients each received 1.0 (alpacas) and 1.5 ml (llamas) GnRH on the same day that the donors was mated. Embryos of both species were collected non-surgically on Day 7.5 post-breeding and transferred to the left uterine horn of a reciprocal female, (alpaca or llama) as described by Sumar et al. (2010). Pregnancy was diagnosed by ultrasound in both species, at 15, and 35 days after ET.

Results

In Group 1, seven of the ten llama recipients that received one alpaca embryo were confirmed pregnant at 35 days post ET (42 days after mating, 70 % pregnancy rate), although one llama aborted at 6 months of gestation. In Group 2, all three alpaca recipients were confirmed pregnant at Day 35 post ET (42 days after mating, 100 % pregnancy rate), but one aborted at 4 months of gestation. The placenta and the aborted fetus of the llama and the alpaca were sent to the microbiology laboratory in order to investigate the cause of abortion; no bacterial or viral infections were found but no yeast cultures were performed.

The average birth weight of alpacas born from llama recipients was 3.3 kg higher than alpacas born from alpacas on the medium-size farms in the Peruvian altiplano and those in our ET Lab (7.0 kg is the average normal birth weight of an alpaca cria born from alpaca dam). However, llama crias born from alpaca mothers had an average birth weight of 10 kg, which is 1 kg less than the average birth weight of llamas born out of llamas. At 6 months of age, the average body weight of alpaca crias born from llamas was 12 kg higher than their counterparts born from alpacas, but at one year of age, both groups of crias, had reached their normal average body weight. In the case of llama crias born from alpacas, they reached their normal weight at 3 months of age. The duration of gestation was similar to that recorded to both species (av. 345 for alpacas and 360 to llamas).

Discussion

Examples of attempted and successful hybridization of species are abundant, but only occasional successes are documented for interspecific hybrids even after several attempts (Gray, 1971). From a practical perspective, interspecific pregnancy is viewed as a tool for preservation of endangered mammals, and the ability to hybridize successfully is probably a dependable indication of compatibility for ET (Anderson, 1988; Klein et al, 1993). Earlier studies done in SAC revealed that crosses between the four species are possible, including crosses between domestic and wild SAC, and the hybrids are fertile (Maccagno, 1932; Argentina, 1940; Gray, 1971; Toledo and San Martin, 1948). Hybrids between alpacas and llamas are very frequent in the small herds in Peru and Bolivia, resulting in the fertile hybrid "huarizo" (Calderon, 1956); also the hybrids between alpacas and vicuñas are frequent, with a fertile hybrid called "paco-vicuña" (Leyva et al, 1977; Sumar, 1992), and hybrids between llamas and guanacos have also been reported (Mac Donagh, 1940). The few reports of hybrids between the two wild species, guanaco and vicuña, came from zoological gardens – but are yet to be confirmed.

The alpaca crias born from llama mothers, were taller than those born from alpaca mother so they were able to reach the udder of the llama dam quite normally. As the llama produces around 50% more milk than the alpaca (Jimenez, 1970; Leyva et al, 1983) these alpaca crias were able to drink at least 50% more milk during the lactation period. This meant that their growth rate was higher, as well their increase in body weight, which allowed them to be more resistant to the environmental conditions during the winter months (May to October), which is the season when the mortality rate of crias is very high (Ameghino, 1991; Ramirez, 1991). It was presumed that the two cases of abortion, in this study, were accidentally induced by transporting the pregnant mothers by truck. The pregnancy rate from interspecific ET was similar to that of intraspecific ET: 56% in alpacas and 72% in llamas (Sumar, 2008). In conclusion, although the use of an alpaca as a surrogate mother for a llama might not necessarily be practical, this study indicates that interspecies embryo transfer is possible in SAC, and confirms the theory of a very close "genetic distance" between the these two domestic species of SAC. The findings also indicate that the alpaca and llama must, have similar glycosylation patterns at the fetomaternal interface but more detailed histological studies of the fetoplacenta interface in the four species of SAC are needed to confirm this.

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Twin reciprocal embryo transfer between alpacas and llamas

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Introduction

Twin crias from alpacas or llamas are very seldom born alive under the natural breeding conditions in the highlands of Peru (Sumar, 1980), and there are no reports of twin parturitions in the wild camelids (Koford, 1957; Raedecke, 1978). Previous reports about llamas and alpacas in USA indicated a very low twining frequency in both species (Fowler, 1990; Sweig, 2002) and similarly, studies carried out in Peru reported low double ovulation rates of only 3 % and 0.9 % after natural mating in alpacas and llamas respectively (Calderon, 1968; Sumar and Leyva, 1979). However, Bravo et al. (2000) reported a high incidence of twin pregnancies (12.5%) in alpacas, which were detected between days 21 and 33 after copulation, but the conceptus identified in the right uterine horn became undetectable between days 28 and 33. Fernandez-Baca et al. (1970) and Sumar, (1980) have also observed twin pregnancies in alpacas between 38 to 42 days after mating with one live foetus being located in the left uterine horn and one dead foetus in the right uterine horn. There are no reports of twin calves in dromedary camels, although some abortions have resulted in two fetuses being expelled (Yagil, 1994) and reports of twinning in Bactrian camels are controversial. In a study of single and twin embryo transfers from Bactrian camel donors into recipient dromedaries, four healthy calves were born but there were no twin births (Niasari-Naslaji et al., 2009). Interestingly, the pregnancy rate following twin embryo transfer seemed to be better than that after single embryo transfer, although the number of transfers were not sufficient for definitive conclusions to be reached. In ewes, Nancarrow et al. (1982) proposed that the greater the number of transferred embryos, the greater the luteal resistance to the luteolytic effect of prostaglandin F2α.

The aims of this study were therefore: 1) to study twin reciprocal embryo transfer pregnancy rates between alpacas and llamas; 2) the proposed hypothesis that the bigger and taller llama has a larger uterus and therefore a better chance of supporting twin fetuses, and 3) the greater the number of transferred embryos, the greater the luteal resistance to the luteolytic effects of PGF2α.

Material and Methods

The study was carried out at the Quimsachata Research Station, Camelid Research Program, INIA, Puno, Peru, which is located at about 4,100 meters above sea level, 55 km west of the city of Puno. The alpacas and llamas used in this study were kept on good quality natural pastures with free access to water. Non-pregnant, non-lactating adult female alpacas (n = 47), and non-pregnant, non-lactating adult female llamas (n = 55) with an average body condition score of 3 (on a scale of 1 to 5) were selected for these experiments. The ovarian activity was monitored daily by ultrasound (ALOKA 500D, with a 7.5 MHz transducer, Japan) and when a dominant follicle (diameter ≥ 7 mm in alpacas and ≥ 9 mm in llamas) was detected in their ovaries, donors from both species were mated and then immediately received a single injection of 1.0 (alpacas) or 1.5 ml (llamas) GnRH (0.0042 mg of buserelin acetate/ ml, Conceptal®, Intervet) intramuscularly (i.m) to aid ovulation. Simultaneously, alpaca and llama recipients were synchronized with the donors by injecting similar doses of GnRH on the same day that the donors were mated. For superovulation donors from both species, with only small follicles and no CL in their ovaries, were selected and injected with either 400 or 800 IU of eCG (Folligon, Bioniche, Canada) for alpacas and llamas respectively. In both species embryos were collected on Day 7.5 post-mating (single and superovulated) and transferred into the left uterine horn of either the synchronized alpaca or llama recipient according to the protocol. Pregnancy diagnosis was carried out by ultrasonography, at Days 25, 35, 38 and 45 post breeding.

A total of four experiments were performed; **Experiment I**, each llama recipient (n=15) received one alpaca embryo from alpacas (n=20) flushed during their natural follicular wave cycle. **Experiment II**, each alpaca recipient (n=14) received one llama embryo that had been collected from llamas (n=22) during their natural follicular wave cycle. **Experiment III**, eight female llamas were superovulated and two embryos were transferred into each alpaca recipient (n=10). **Experiment IV**, six female alpacas were superovulated and two embryos were transferred to each llama recipient (n=7).

Results

In Experiments I and II, the embryo recovery rates from single ovulation uterine flushes were 70% and 80% for alpacas and llamas respectively. The average number of embryos recovered from superovulated llamas was 3.5 embryos per female (4 small embryos were discarded), and in alpacas the average recovery rate was 4 embryos per female (10 embryos were discarded for poor quality).

The pregnancy results for each experiment were as follows: Experiment 1, alpaca embryos into llamas resulted in a 53.3% pregnancy rate. Experiment II, llama embryos into alpacas resulted in a 42.8% pregnancy rate. Experiment III, twin llama embryos into alpacas resulted in 70.0 % single pregnancies, and two alpacas conceived twins (one embryo in each uterine horn) but the fetuses in the right horn died at 42 and 45 days post-mating. In Experiment IV, twin alpaca embryos into llamas resulted in 71.4 % single pregnancies, but one fetus died at 45 days of gestation. There was no statistical significant difference between Experiments I and II ($P \leq 0.05$), or between Experiments III and IV ($P \leq 0.05$). However, there was a significant difference in pregnancy rate, between Experiments II and IV ($P \leq 0.01$), in favor of twin embryo transfer. No live twins were born in Experiment III and IV.

Discussion

The embryo recovery rates in both species were similar to those obtained in other studies carried out in different countries under different management systems (Del Campo, 1995; Huanca et al, 2007; Miragaya et al, 2006; Sumar, 2008). The new superovulation protocol using eCG in low doses in alpacas and llamas that had no mature or active CL in their ovaries, yielded a moderate number of embryos. The protocol using lower doses of eCG had the added advantage that it did not change the anatomical and physiological structure of the ovaries and thus problems of bleeding, rupture of the infundibulum and adhesions with other surrounded organs than can result from using higher doses of hormones, did not occur (Sumar, in press). Results from Experiment I and II, are in agreement with results presented in this Camelid Satellite Meeting (Sumar et al., 2012). The natural and artificial interbreeding of alpacas and llamas, tells us about the very close "genetic distance" between these species and, the similarity between their placentas, and glycosylation patterns at the fetomaternal interface. The pregnancy rate of Experiments II and IV (42.8% vs.71.4 %), favors twin transfers and the luteothrophic effect of twin conceptuses on the uterine environment during early pregnancy (12 to approximately 38 days). However as the twin conspectuses develop beyond 38-40 days they compete for space within the uterus and therefore the right horn fetus is frequently aborted.

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Factors affecting embryo recovery in dromedary camels: Review of results over the last 20 yrs

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Introduction

Breeding replacement racing camels is always a slow process under traditional natural mating conditions. The long gestation averaging 385 days (Manefield and Tinson 1996) means a calf is at best produced every 2yrs, and with the better performing female camels often racing until at least 10-12 yrs of age the length of time to produce offspring is limited. The development of embryo transfer has given the opportunity to produce multiple calves with multiple sires in a single season via the use of superovulation and surrogate mothers (McKinnon et al 1994, Tinson and Sambyal 2008). Certain factors including donor selection, superovulatory treatments and the bull camel management are central to producing good results. This paper summarizes the major factors involved in trying to optimize embryo recovery and how these influences may have changed over the period since E.T. was first pioneered in camels.

Materials and Methods

The process of embryo transfer in the camel is well documented (Tinson et al 1998) and the basics of the process have remained relatively the same. Camels are examined in a restrained standing position with ultrasonic examination being central to all decisions in regards to time of mating, selection of donors and recipients and monitoring of hormonal responses.

Over the last twenty years pregnancy rates have improved from an average of 32% (McKinnon et al 1994) to 67% (Tinson and Sambyal 2008) but from year to year there are variations in average collection rate and total pregnancy numbers depending on availability of suitable donors and bulls. Our results have been reviewed from the early days 1990-94 (collections carried out 7 days post mating), through to when pregnancy and collection rates started to approach the best results in 1995-97 (changing from Day 7 to Day 9 collections post mating) and then to recent times 2010-12 (almost exclusively Day 9 collections).

The factors that have been focused on are those that most consistently influence the result, which are: age and reproductive status of donors, selection and management of bulls, choice of superovulatory treatment, time of collection and number of times collected.

Results

Age and Reproductive Status of Donor

1990-94-Embryo recovery from donors <12yrs (299/124, 241%) was similar to that from older donors ≥12yrs (226/86, 263%). More embryos were obtained from parous females (322/84, 383%) than from maiden/nulliparous females (203/126, 161%).

1995-97- Embryo recovery from donors < 12yrs: 342% (558/163); and donors ≥12 yrs: 378% (265/70) were not significantly different (p=0.191). However there was a significant difference with regards to parity: calved donors (parous) 394% (552/140) and maidens 302% (281/93; p< 0.0001).

2010-12-in the later years we have concentrated on recently retired champion camels so very few are over 12yrs in age. Embryo recovery from donors <12yrs: 391% (348/89) and camels ≥12 yrs 150% (9/6). Parous camels were 436% (192/44) with maidens 321% (165/51).

Selection and Management of Bulls

It is difficult to compare individual bulls over long periods of time but trends in individual ability to fertilise oocytes will be directly related to the quality of their semen. While bulls can be successfully used a number of times a day to produce individual pregnancies in a normal mating programme, producing larger numbers of embryos from superovulated individuals is a different proposition. When trying to fertilize a larger number of oocytes from a single donor, the length of rest prior to mating plus the number of matings become critical. These factors will influence how vigorous the mating is and the amount of sperm delivered. In an induced ovulator such as the camel this will have a significant influence on how well ovulation occurs. The ability of the camel to fertilize oocytes, its "strike rate," is important and often fairly consistent (Tinson and Sambyal 2008).

1990-94- In one season a bull produced no embryos from 10 superovulated females whilst another bull produced 31 embryos from 11 donors. The poorly performed bull was electroejaculated and found to have low total sperm numbers (only 80 million) and a low proportion of progressive motility (<10%), yet had testicles of normal size and consistency.

1995-97- Males varied from Misaihan with a high of 59 embryos from 7 matings (843%) to Zafran with low of 0/4. The more popular bulls at the time showed more consistent results from season to season. Mileh Sageer produced 143 embryos/32 (447%) in 95/96 followed by 64/14 (457%) in 96/97, a very consistent average of 450%. Another well used bull Thabiyan gave 83/15 (553%) in 95/96 and 48/7 (686%) in 96/97 an average of 595%. Out of 12 bulls used, 8 managed an average of >250% recovery. Sadeeh at 20yrs old was well performed on progeny tests in terms of champions produced but his average recovery was only 38/17 (224%) over both seasons. Arthritis and lower sperm numbers would influence these results.

2010-12- In these seasons the number of embryos/ preparation and "strike rate" i.e. embryos / corpus luteum were examined.

The embryos/preps, gave the following results over the two seasons. Nashwan 144e's/30 preps (480%), Mias 113/39 (290%), Lasaad 34/6 (566%) gave good results but Mustail 13/5 (260%), Mutafi 7/3 (230%) Tagi 4/2 (200%) and Khartoum 0/4 were less good. However embryos vs preparation doesn't allow you to predict what might be recovered on an individual collection and lack of ovulation could make a bull appear to perform poorly. Looking at the "strike rate" results for the same bulls over the two seasons gave the following results: Nashwan 144 e's/198 CLs (16.45%), Mias 113 e's/875 CLs (16.4%), Lassad 34/198 CLs (17.2%), Mustail 13e's/136 CLs (9%), Mutafi 7 e's/96 Cls (7.3%), Tagi 4e's/55CLs (7.2%) and Khartoum 0/67.

Selection of Superovulatory Treatment

1990-94- more embryos were collected from camels treated with FSH (Ventrepharm Folltrophin) and naturally mated 384% (261e's/86 preps) compared to eCG (Pregnecol Horizon) 230% (193/84). With artificial insemination (A.I.) FSH gave 188% (49/26) and eCG (Pregnecol) only 69% (22e's/32 preps).

1995-97- Average over the two seasons showed FSH (Folltrophin) giving significantly higher recovery rates compared to 1990/94 with 423% (567e's/134 preps) and higher than eCG (Pregnecol-PMSG) for the same period at 268% (266/99) ($p<0.0001$).

2010-12- FSH (Folltrophin) showed a lower level of recovery with only 242% (126 e's/52 preps) and eCG (Folligon-Intervet) 342% (212/62).

Time of collection

1990-94- All camels flushed 7 days post mating with reflushes sometimes at 7.5 days.

1995-97- Over the two seasons camels flushed 7 days post mating resulted in 599 e's/149 preps (402%) vs 9 days post mating which resulted in 166e's/ 50 (332%) (p=0.30). The number of donors that produced embryos on Day 7 was 79% (117camels /149 flushes) vs 78% flushed at 9 days (39/50).

2010-12-All camels were flushed 9 days post mating. Recovery rates were 338e's/114 collections (296%)

Number of times donor collected

1990-94- No apparent adverse affects of multiple collections. Two camels were collected 5 times, and 14 were flushed 3 times. Higher producing donors at first flush tended to remain high producers in following flushes especially if they responded well to FSH. Embryo recovery did tend to decline in donors repeatedly treated with gonadotphins. Giving donors a season off with a natural pregnancy appeared to decrease the decline.

1995-97- Repeat flushes were common in these two seasons. Donor camels were often used 3 consecutive times in a season. Of 5 particular camels flushed 3 times in the 96/97 season 3/5 had their best collection in the 3rd program, Mattara- 0, 1 and 11 embryos, Shrood- 15, 7, 3, Al Zahrah- 0, 3, 11, Misaiha- 0, 27, 2 and Nowia- 0, 8, 9.

While generally PMSG tends to see a decreasing level of embryos one camel Bint Mileh on Pregnecol gave 6, 28, 5, 6 then when changed to FSH gave 13 embryos. Another Khadathera went consecutively FSH/1, PMSG/3, PMSG/4, FSH/19, and then PMSG 10 with donor also pregnant.

2010-12- Camels with 1-2 preparations only Folligon (Intervet) 140/28 (500%) but FSH (Folltrophin) was only 326% (75/23). Camels with 3 previous preparations or more (up to 12) Folligon (Intervet) 211% (72/34) and FSH 176% (51/29)

Discussion

In terms of results there were no significant differences in collection timing between 7 and 9 days post collection but the 9 days does offer more efficient working schedules (no reflushes, more efficient recipient use) as well as better pregnancy rates. In 96/97 when comparing 7 to 9 days directly we got 37 pregs/92 transfers (40%) whereas Day 9 gave 62 pregnancies from 114 transfers (55%) p=0.050. Day 9 transfers peaked at 67% (Tinson et al., 2007) with 19/24 tranvaginal transfers resulting in pregnancy (79%) and 12/22 transrectal pregnancies (55%). With increased pressure on number of embryos collected and being transferred, and with donors tending to be recently retired nulliparous females, the collections

rates are decreasing and maiden recipients (poorly performed race retirees) are coming more into use.

Overstimulation of maidens may in fact create anatomical issues with overly large superovulatory responses "herniating" out of the ovarian bursa and affecting oviduct pick up. In 2006 we changed from using Pregnecol (4,500IU) to Folligon (5,000IU) as an alternative to FSH. Better than expected follicle development occurred but a poorer percentage of embryo/follicle were collected. In 1998, 47 preparations of eCG (Pregnecol) resulted in 491 follicles (1,044%) with 152 recoveries (323% -either embryo, UFO-unfertilised egg or donor pregnancy) with recovery/foll (14.5%). Only 5 of the 47 (9.4%) preparations gave more than 20 follicles. More than 12 yrs later eCG (Folligon-Intervet) over the two seasons 2010-12 where 76 preparations were carried out, a total of 1999 folls (2630%) were produced with 265 recoveries (348%) but recovery/follicle was only 10%. In this period 40/76 preparations gave 20 follicles or more (53%) and 20 camels gave 40+ follicles (26%).

The bull factor is vital in trying to optimize recovery (Tinson and Sambyal 2008). The bulls "strike rates" quoted were in the middle of performance ranges but the owner's personal preference often determines the matings. Some individual bulls consistently produce high embryo recoveries: Shaheen Khilaili had a strike rate of 25%, Saraj 52% and Mashcour 50%. Once Mashcour mated a camel with an ultrasounded count of 28 follicles on both ovaries to produce 29 embryos! Nashwan when used sparingly in previous seasons had a strike rate of 38% (51 embryos from 133 ovulations in 9 matings) but recently (2010-12) he was used 30 times and only managed a 16.45% "strike rate (144 embryos/198 ovulations). Overuse of superior bulls is a big issue and potentially reduces the "performance" of a good E.T. programme.

Conclusion

When the veterinarians are allowed to make informed decisions on donor and bull selection related to superovulatory response it is possible to push embryo recovery averages to around 5 embryos/ donor and above. However the pressure to repeat the use of superior individuals, with over use of males and repeat flushes of females without allowing a "rest" through a year off (with a normal pregnancy) can dramatically decrease recoveries and thus potential pregnancy results.

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Interspecies embryo transfer: A suitable approach to conserve Bactrian Camels

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Introduction

It is assumed that the Bactrian camel was domesticated on the eastern border of the Caspian sea around 2500 BC, and from there it migrated to several countries worldwide (Niasari-Naslaji, 2008). In Iran it has acclimatized to the cold environment but is threatened with extinction. Unfortunately due to several factors including changes in transportation, domination of dromedary camel and inability of the Iranian Bactrian camel to acclimatize to a hot and arid environment, the population of this species has decreased to about 156 animals in Iran. The purpose of this research was to conserve and extend this species to an arid and hot environment through interspecies embryo transfer.

Materials and Methods

Bactrian camels (donors) belonging to the Bactrian Camel Research Center, Meshginshahr, Ardabil Province in Iran and dromedary camels (recipients) from the same research center as well as the Camel Breeding Center for North and North-West Iran, Toroud, Semnan Province and one private camel farm in Qom Province of Iran were used in this study.

The female Bactrian donor camels were superovulated according to the procedures described previously by Nikjou et al. (2008). In brief, two days after inducing ovulation of a mature follicle using a single injection of GnRH agonist Alarelin (25 µg, i.m., Vetaroline, Aburaiohan, Iran), the female was injected with decreasing doses of FSH (60, 40, 30, 30, 20 mg, i.m.; total 380 mg; Foltropin-V; Bioniche, London,ON, Canada) twice daily for 5 days, followed by a single injection of 20 mg FSH, i.m., on Day 6. Prostaglandin F2α analogue was injected on Day 5 of superovulation. Daily ovarian ultrasonography was performed until most of the growing follicles had reached a size of 10–17mm. At this time, the donor camel was mated twice, 24 h apart, to a fertile male Bactrian camel and given an injection of GnRH

agonist (i.m.) at the time of the first mating (Moghiseh et al., 2008). Embryos were then recovered 8.5 days after the first mating.

Recipient dromedary camels were synchronized using two injections of GnRH agonist, 14 days apart (Nikjou et al., 2008; Nikjou & Niasari-Naslaji, 2010). Ovulation was then induced when a mature follicle of 13–17mm in diameter was detected in the ovaries and embryo transfer was performed 6.5-7.5 days after GnRH injection.

The uterus of the donor Bactrian camel was flushed non-surgically 8.5 days after the first mating using a silicone two-way Foley catheter (Length 64 cm, 20 Fr; AB Technology, Dresher, PA, USA) and a total volume of 2 L Ringer's solution (Iranian Pharmaceutical Products, Tehran, Iran) containing 0.2% bovine serum albumin (BSA; Sigma, St Louis, MO, USA) or 2.5% camel calf serum. Recovered embryos were transferred into holding medium (ZA454; IMV Technologies, L'Aigle, France) and were washed four times with fresh media before morphological evaluation under a stereomicroscope (Olympus SZX12; Olympus, Tokyo, Japan). Embryos were graded from 1 to 5; Grade 1, excellent; Grade 2, good; Grade 3, poor; Grade 4, collapsed and degenerated; Grade 5, fragmented and degenerated. For embryo transfer, only Grade 1 embryos (in pairs) were loaded into 0.25-mL straws (IMV) and transferred non-surgically into the uterine horn of the dromedary camel recipients using a bovine/equine embryo transfer gun (IMV). The presence of an amniotic fluid filled sac and a CL was diagnosed on Day 14 after embryo transfer (Day 21 of gestation) using ultrasonography and pregnancy was confirmed on Day 25 by the presence of a fetus with a heart beat.

Results

The first male Bactrian camel calf resulting from interspecies embryo transfer was born in 2008 (Niasari-Naslaji et al., 2009), and since then another 14 calves (5 males and 9 females) have been born. Nine calves were born at the Bactrian Camel Research Center, 5 calves were born at Camel Breeding Center and 2 calves were born at the private camel farm in Qom Province of Iran. At the moment, they are between 1.5 - 4.5 years of age without any particular problems. Four calves will be ready for mating in the breeding season of 2012.

Discussion

In this project, superovulation and embryo recovery was successfully conducted in the Bactrian camel and the dromedary camel has proven to be an excellent recipient for Bactrian camel calves. Dromedary camels have good mothering abilities and produce more milk than

Bactrian camels resulting in good growth rates of Bactrian camel calves. Using interspecies embryo transfer, we have demonstrated for the first time that Bactrian camel calves, born from dromedary camels in a hot environment, in which the temperature can rise to 50˚C, can tolerate a hot environment. This novel finding will allow us to extend Iranian Bactrian camel into hot climates as well as cold. In conclusion, interspecies embryo transfer opens a new horizon for preserving Bactrian camels from the threat of extinction.

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Observations on embryonic loss and abortion in racing camel (Camelus Dromedarius) breeding programmes

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Introduction

With the value of elite racing camels valued often in millions of dollars breeding replacement animals is a vital part of the management of a successful racing team. While embryo transfer is a well documented part of this process (McKinnon et al 1994, Tinson et al 1998), the loss of pregnancy during the long gestation represents significant frustration and economic loss to those involved with the process.

There have been varying reports over the last 20 yrs on reproductive loss in the camel from a number of authors. Wilson (1984) reported calving rates with normal husbandry techniques at around 40%, Rai et al (1991) found very high levels of embryonic resorption and foetal losses (75%) on the first cycle of matings of camels in India where puberty had been induced early using gonadotrophins. Tibary et al (2001) mentioned early loss figures in dromedaries of 18% and Skidmore et al (2005) also reported losses of 18% (4/22) in a group of E.T. pregnancies.

Materials and Methods

Our records have been reviewed back to the early 90's to look at the levels of pregnancy loss, focusing on the years where there were accurate records on when the losses occurred and the corresponding births.

Camels were checked by ultrasound for pregnancy at around 20-22 days (11-15 days post transfer depending on age of collection of embryo) and then groups of pregnant camels were monitored every 2 weeks for the first few months and then monthly till term. This was achieved using male camels to move amongst the pregnant females to provoke the female camel's unique tail lifting response (Wilson 1984, Abdel Rahim 1992). Any suspect losses then re-checked by ultrasound.

Results

Pregnancy losses varied considerably from season to season. In seasons 95/96 and 96/97 the best figures were achieved with an average of just 14% of recipient pregnancies being lost (37/256). However, in stimulated donors over the same two seasons there were 40% (17/42) losses in donor female pregnancies that were either not flushed post mating due to poor response, or had got pregnant subsequent to flushing.

The following season 97/98 losses were considerably higher with 100 births from 161 pregnancies of which donor losses represented 15/35 (43%) and recipients 46/126 (36.6%). Within this season there were 3 collection programs (E35, 36, 37) with the best individual results being donors in E37 80% births (12/15) and recipients in E35 67% births (21/31). The worst results for donors and recipients were in E36 with only 5 births from 9 pregnancies (56%) from the donors and only a 60% calving rate (32/53) from recipients.

More recently in the 2010-11 season 101 recipient pregnancies resulted in 61 births. Out of the 40 pregnancies lost 14/40 (35%) occurred in the 1st 40 days, only 3/40(7.5%) from day 41-60, another 12 (30%) from day 61-80, 2/40 (5%) from day 81-100, 4/40 (10%) from 100-150 days, 1/40 (2.5%) days 151-200 and a further 2/40 (5%) day 201-300. During the season 2011/12 a total 107 recipient pregnancies were achieved and so far 34 have lost. Of these 12/34 (35%) lost during the first 40 days, 18/34 (53%) between days 41-60 and 4/34 (12%) between days 61-80. These results represented losses prior to 60 days of 42.5% in 2010/11 but over 80% in 2011/12.

Surprisingly neither viral nor bacterial infection seems to have played a significant role in influencing the E.T. pregnancy or loss results. The camels unique immune system seems to play an important role here.

Discussion

There were significant variations between groups over the five seasons studied where more accurate data was available. These figures are currently being compared to those of naturally mated animals with no gonadotrophin stimulation. In some smaller individual groups it would appear to be similar but we are currently observing a group of 150 natural pregnancies to check the situation in a larger group. In bovines, King (1985) compared abortions rates in non-stimulated (10/136 - 7.4%) and stimulated females (84/1640-5.2%) with no significant difference (p=0.66). Those loss rates are low compared with our results, however bovine pregnancies at that time were not checked until around 40 days and at this point the camel has conceivably already lost 35% (2010-11) of pregnancies. King et al (1985)

evaluated embryonic losses and abortion in his large study of bovine pregnancies and reported a loss of 3.15% between 2-3 months and 2.14% for 3-7 months. However when premature birth, dystocia and recipient death was added, the figure increased to 14 %.

Comparative losses in high producing dairy cows in A.I. programs have been reported at varying levels with losses between 25% from Day 21 to Day 80 in Holland (Grimaldi et al 2006) and 7.2% between Day 28 to 84 by Silke et al.,(2001). These also featured almost half (47.5%) of the losses occurring between Days 28-42. Embryo quality (including recessive defects affecting embryo survival), age of mother, as well as negative energy balance in high producing animals are all shown to have an effect on embryo survival. There are some indications that some of these animals could have increased expression of many key genes know to affect inflammatory responses (Diskin et al 2012).

A number of small pilot studies have been initiated to try and look at factors that could influence these pregnancy loss figures. Vitamin E deficiency has been reported regularly as causing disease in camels in the Middle East and elsewhere (Manefield and Tinson 1996). We have regularly found deficient levels of Vitamin E in pregnant camels as well as new born and lactating females in Abu Dhabi. Recent random sampling of 20 breeding females fed on Rhodes grass hay with no supplementation showed an average Vit E level of 0.73mg/L (ref range 1.5-2.5 mg/L), Vitamin A av. 0.24mg/L (0.32-0.45mg/L) and Vit B1 av. 68.75ug/L (28-85 ug/L). Experiments to compare embryonic loss in pregnant animals both supplemented and non-supplemented with Vitamin E prior to and during pregnancy have so far failed to show a difference. Comparision of cortisol levels and progesterone in early pregnancy have failed to elucidate any clear connection to loss in the camels despite “stress” clearly being important with loss of corpus luteum. There is no doubt that stresses such as transport, nutrition and season during pregnancy could play a role.

Omega-3 has also been implemented with pregnancy and fertility in different domestic animals and humans (Gulliver et al 2012). A pilot study involving 20 pregnant camels to assess its influence on embryonic loss has been carried out recently but there were no significant differences between groups studied to date. On the nutritional side an improvement was noticed when the protein content of the diet was decreased when camels were diagnosed pregnant and it was kept low for at least the first few months.

Seasonal changes also need to be studied re average temperatures vs rainfall, sand storm incidence and humidity to see if they play a role in influencing seasonal figures of pregnancy loss. In Program E 84 during the 2011-12 season there was a particularly high early loss of pregnancy in a group of Hasmi beauty camels transferred to Pakistani Camel

recipients. A total of 21 transferred embryos resulted in 14 pregnancies but by 40 days only 8 pregnancies remained intact representing a loss of 43%. During this period there were two very severe sandstorms which both lasted a number of days, which does make one question the potential influence of season and stress. The kangaroo (also an induced ovulator) is able to manipulate its cycle and pregnancy to respond to seasonal stress with embryonic diapause. Is it possible that the camel takes a more aggressive (terminal view) of pregnancy under seasonal stress. Given their desert surrounding being generally defined by variable food and water resources, rather than an embryonic diapause mediated by prolactin (Mead 1993), it could be total inhibition and regression of luteal activity causing actual regression of corpus luteum via cortisol and other hormones.

With regard to possible infective reasons being the cause of losses that may go undetected, we found in the 1995-97 period that when embryonic losses occurred the recipients were checked via ultrasound and successfully recycled to later programmes with good subsequent pregnancy rates. Camels recycled from season 95-96 had a pregnancy rate of 47% (29 preg/62) and some camels that received 4 preparations in a row (due to early loss of pregnancy or failure to get pregnant) their pregnancy rate after the 4^{th} preparation was still 49% (20/41) which was similar to first time transfers at the time 49% (113/230) (p=0.82).

Conclusion

Embryonic loss occurs at a high level in camel breeding programs whether using assisted reproduction or natural mating. More extensive studies are necessary to determine whether these loss rates can be influenced via management, nutritional and hormonal means or whether high embryonic loss is just a normal phenomenon in the breeding of racing camels.

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Cloning by somatic cell nuclear transfer in camels: Cytoplast source influences the development of reconstructed embryos

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Since the production of first cloned mammal by somatic cell nuclear transfer (SCNT) in 1996 (Campbell et al., 1996), cloned offspring have been produced successfully in many species including the camel (Wani et al., 2010). Cloning by SCNT has a special significance in the genetic improvement of camels and can be used to produce animals with the highest potential for milk production, racing, males of high genetic merit or the beautiful camels called "Beauty queens". Optimization of the techniques for *in vitro* oocyte maturation (Wani and Nowshari, 2005; Wani and Wernery, 2010), *in vivo* oocyte maturation and ultrasonographic guided ovum pick up (Wani and Skidmore, 2010) chemical activation of mature oocytes (Wani, 2008), and *in vitro* embryo culture (Wani, 2008; Wani, 2009) during the past few years lead to the birth of the first cloned camel (Wani et al., 2010). The potential applications of somatic cell nuclear transfer in camels are currently, however, constrained by low pregnancy rates from the transferred reconstructed embryos. Many factors including recipient cytoplast source, their preparation, nuclear donor cell and their treatment influence the success of the nuclear-transfer process. Little is presently understood of the fundamental molecular and cellular events that could be involved in reprogramming the nucleus of an adult somatic cell. In the present study influence of cytoplast source on the development of reconstructed embryos after nuclear transfer was evaluated.

The mature oocytes were either obtained from super-stimulated donors after an injection of GnRH 26 hours before ovum pick-up, as described previously by Wani and Skidmore, (2010), or from the cumulus oocyte complexes of slaughterhouse origin matured *in vitro* for 28 hours. The skin fibroblast cells obtained from an adult dromedary camel were used as donor karyoplasts after serum starvation for more than 72 hours. Reconstructs were activated 1 h post-fusion and cultured at 38.5^0C in an atmosphere of 5% CO2, 5% O2, and 90% N2 in air. The proportion of oocytes that cleaved was recorded on Day 2, and those that reached morula and blastocyst stages were recorded on Day 7 of culture.

The proportion of successfully fused cytoplast-donor couplets did not differ between the groups utilizing *in vivo* matured oocytes obtained by OPU and *in vitro* matured oocytes from slaughterhouse as donor cytoplasts. However, a reduced number of these couplets cleaved in the group utilising oocytes from slaughterhouse origin compared with the oocytes obtained by OPU. The proportion of blastocyts obtained from the reconstructed embryos was also lower in the group utilizing slaughterhouse oocytes as recipient cytoplasts when compared to the reconstructs from oocytes obtained from live animals by OPU. To the best of our knowledge, there are no previous studies where the development of *in vivo* matured oocytes have been compared with *in vitro* matured oocytes in camelids, after nuclear transfer. However, these results agree with previous studies where the developmental response to chemical activation of *in vivo* matured oocytes collected by OPU was also better than *in vitro* matured oocytes collected from the ovaries of slaughterhouse origin (Wani and Skidmore 2010). Similarly, in cattle the developmental potential of *in vivo* matured oocytes has been reported to be twice that of their *in vitro* counterparts from abattoir origin (Van de Leemput, 1999). The reasons for decreased developmental potential of *in vitro* matured oocytes from slaughterhouse ovaries could be attributed to source and the conditions during maturation of these oocytes. The oocytes collected by OPU were from preovulatory follicles of live animals after several days of treatment with FSH, while *in vitro* matured oocytes were collected from 2- to 10 mm follicles from the ovaries of abattoir origin. It is well documented that the role of FSH in the acquisition of developmental competence is primarily associated with its effect on follicular growth, as several days of treatment are required to obtain oocytes of higher competence. The ovaries from the slaughtered animals come from a heterogeneous group; they are old, unproductive or very young and have not attained maturity. The oocytes from these ovaries do not undergo normal preovulatory development such as selection and growth, which are accompanied by a change in pulsatile release of LH and FSH.

In conclusion, even though the developmental potential of nuclear transfer embryos reconstructed from the oocytes collected from live animals was better than in vitro matured oocytes obtained from slaughterhouse ovaries, both types of oocytes can be utilized as donor cytoplasts and have the potential to develop to blastocyst stage. The ovaries from slaughterhouse are the cheapest source of oocytes and could be utilised for developing the nuclear transfer technology in countries where a good number of camels are being slaughtered daily in organised slaughterhouses.

Acknowledgements

The authors are grateful to Mr. M. Billah, Mr. Aijaz Hussain and Mr. A. N. Siddiqui for their help in handling and treatment of animals and also during the collection of COCs. We also thank Dr. Ali Redha, administrative director, for his support. This study was kindly sponsored by H.H. General Sheikh Mohammed bin Rashid al Makhtoum, Ruler of Dubai and Prime Minster of UAE.

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The Raigi breed! Newly found dromedary camel of Pashtun region

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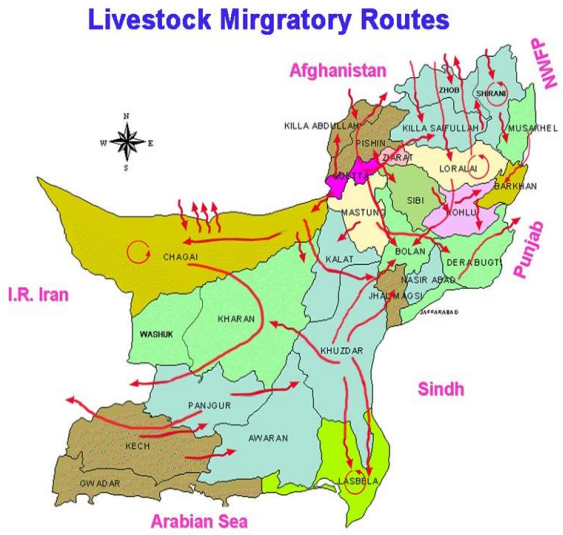
Introduction

The Raigi camel is predominantly found in the historic Khurasan region of Afghanistan, (see Maps 1 and 2) but is also found in some parts of Balochistan. This breed is well adapted to the climatic extremes and is greatly appreciated for its' significance in the pastoral economy. It is mainly raised under nomadic and transhumant types of production systems, where some sedentary people only keep the male animals for working, however some also keep she-camels for milking. The transhumant or semi-nomadic people travel inside the Khurasan region, either in Pakistan or Afghanistan (shown in map 2 below), but some families travel up to the Musakhail district of Balochistan province in winter. In Khurasan, the camel is used mainly as a pack animal by the pastoral people, who travel with their families along the Durand Line and stay near Kandhar, Hirat, Farah, and the Kurrum area of Chaghai district in Balochistan. The sedentary people mainly use Raigi camels for milk and local transportation.

Map 1: Areas where Raigi Breed Found

Map 2: Migratory Routes of Balochistan



Pastoral people have developed and preserved unique breeds of livestock and have developed a lot of traditional knowledge associated with them. Their pastoral lifestyle has

developed the co-evolved ecosystems which they have traditionally conserved and sustainably used. In the Khurasan region the camel is used in religious rituals like Eid-ul-Azha and Sadaqa. Their spiritual universe is linked to their livestock breeding, and their ethnicity is inextricably intertwined with the animal breeds and way of life. This means that their livelihoods and the survival of their particular breeds are based on access to the lands and forests. In return, their animals help conserve the biodiversity of the local ecosystems as they graze them within the regulations of the (Pashtun Biocultural Community Protocol of 2010). The meat of the camel is traditionally dried (Landi) and used during the winter but in recent years the importance of the camel has increased many fold due to various factors such as drought, high male calf prices, demand in the neighboring countries, and awareness of camel products (Raziq et al, 2011).

The pastoral people know the important and salient traits of a local breed, so the breed must be characterized in their own perspectives. First, the socio-economic circumstances in which the breed exists must be documented (Köhler-Rollefson, 2005) and this socio-economic data is also helpful for separating the breeds (Simianer, 2005). The characterization of a breed comprises performance traits, adaptation traits, special traits like drought and disease tolerance and biomolecular studies (Grund, 2004). Local breeds of camels can be categorized similar to cattle into beef, dairy, dual-purpose and racing (Wardeh, 2004), although camels are very rarely reared just for their meat and racing camels are consistent with other camel breeds (Payne and Wilson 1999). For documentation of camel breeds in India, information about socio-economics, habitat ecology, physical characteristics, productivity, management and production systems have been included (Köhler-Rollefson and Rathore, 1996).

The dromedary camel is one of the major livestock species reared by Pashtoon pastoral people. The Raigi camel breed is found in the Kakar Khurasan region of Killasaifula, the Zhob districts of northeastern Balochistan and the nearby Afghan provinces of Paktika, Ghazni, Zabul and Qandharand, and was previously used for salt transportation (see Map1 below). It is well adapted to the ecosystems and climatic extremes, the brackish water and the salty vegetation and is therefore significant to the pastoral economy (Raziq and Younas, 2006) and socio-economic culture (Knoess, 1977). It also represents an indigenous animal genetic resource.

Aim

The aim of this study was to characterize the Raigi camel breed in the context of habitat ecology, socio-economics, management and production system, and to evaluate its significance for the future.

Materials & Methods

Information was collected by the author from 32 camel herders during 2007 and 2008. Physical measurements of the various camels were carried out by hand as described by Schwartz and Dioli (1992) and Grund (2004).

Results& Discussion

Physical characteristics

The color of the Raigi camel changes from fawn in the summer to brown in the winter. The camel has long eye lashes, dark brown retina and black toe nails as well as a large and barrel like body, which indicates its dairy potential. Body measurements of the Raigi camel are presented in Table 1.

Table 1: Biometric parameters of Raigi breed

Body measurements	**Male**	**Female**	**Mean**
Head Length	39.48	39.19	39.25
Head Width	20.14	19.75	19.87
Wither Height	165.67	163.93	164.34
Thoracic Girth	175.33	173.5	174.415
Abdominal Girth	210	237.3	223.65
Tail Length	49.10	47.21	47.67
Ear Length	11	10.99	10.99
Ear width	5.95	5.99	5.98
Neck Length	82.76	76.14	78.49
Body Length	139.33	139.27	139.3
Estimated Weight	337.58	384.90	439.995

Productive and Reproductive Performance

Breeding starts at the onset of puberty for both males and females, and the breeding season runs from November to January. The period of receptivity in the female (as they are induced ovulators) ranges from 1- 4 weeks and one vigorous bull can serve up to 40 females.

The service period lasts for 5 to 6 days, and if she conceives, the female changes her behavior on the 6th day of service by erecting her tail when an animal or person comes near her. The calving interval depends upon the availability of foliage and lactation length, and birth weight of the calf is dependent upon the sex, as well as nutritional and health status of the dam. Weaning occurs at 9 months of age. According to the Raigi camel keepers, the special traits of the breed are that they produce thicker milk compared with other camels, and have the ability to drink brackish water and eat the bitter plant *Artemisia*. The milk is salty because they graze entirely on *Haloxylon* and *Artimisia*. The wool has a long staple length with fine fiber and is mostly used locally for making rugs. The major breeding goals for the camel keepers are a consistent production of thick milk over a long period of time, good climbing ability in mountains (wide chest and thick cannon bone) and also good endurance over long distances. Reproduction and production data are presented in Table 2.

Table 2: Productive and reproductive performance of Raigi camel

No	**Traits**	**Values**	
		Male	**Female**
1	Average birth Weight	30 kg	33 kg
2	Average weaning Weight	140 kg	160 kg
3	Ready for workload	3 yrs	3 yrs
4	Use for heavy duty	7 yr	___
5	Age of puberty	3.5 yrs	3 yrs
6	Average work life	20 yr	___
7	Average reproductive life	15 yrs	21 yrs
8	Conception rate out of herd	___	50-53%

Conclusion

The Raigi camel represents a valuable genetic resource for present and future food production so immediate measures are necessary to ensure its survival as a separate gene-pool. These measures include the conservation of the habitat ecosystem and the recognition of the rights of the Pashtun pastoral people in making their living from keeping livestock in the Khurasan region.

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Genetic characterization and differentiation of Camelus dromedarius of Iran

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Introduction

Animal production plays an important role in quality of products produced. However if production could be carried out under less intensive conditions it would ensure more respect for the environment, give guarantees for the sustainability of the system and help to maintain the population in the rural areas. Within these strategies it is desirable on the one hand to maintain biodiversity preventing the loss of genetic patrimony and on the other hand, to preserve local breeds to help the subsistence of rural populations (Paredes et al. 2011).

The reduction in stock sizes of endemic species continues to be a conservation concern in many regions of the world. Livestock management activities have relied on describing the existing genetic attributes of the remaining populations. These include incorporating this information into managing phenotypic characteristics, performance, quantitative trait loci (QTL) development, cultural importance and preserving the genetic uniqueness of the remaining populations (Duchev and Groeneveld, 2006).

In Iran the majority of camels are dromedary (or single-humped) camels. They play an important role in the establishment of the extensive agriculture system, and the production of camels is the main economic activity of the residents. However, with the advent of motor vehicles camels have been superseded and many were simply released into the wild. In total there are now approximately 140,000 dromedary camels in Iran. The estimation of genetic variability and the relationship between different livestock breeds is important for management of genetic resources for their sustainable utilization and conservation. Utilizing microsatellite markers for the estimation of genetic diversity and relationships amongst livestock species is well known and recommended by the Food and Agriculture Organization, International Society for Animal Genetics (FAO-ISAG), so is ideal for such studies (Hampton et al., 2004). However there is no such information available on the genetic

diversity of the herds of camels in Iran. Therefore, this study was planned to investigate genetic variation in Iranian camels using eight microsatellite loci.

Material and Methods

We have analyzed 150 individual dromedary camels in three populations from three different geographic regions (yazd, semnan and shahrod) of Iran. Samples were taken from the jugular vein and DNA was extracted by RBC mini kit (Cat No. YGB100) for mammalian blood. Eight microsatellite markers (Table 1.) were amplified by multiplex PCR methods and analyzed in all samples.

Table 1: The primers used for amplified microsatellite loci

Primers	Forward	Backward	Reference
Primer1	GAAGAGGTTGGGGCACTAC	CAGGCAGATATCCATTGAA	Spencer and Woolnough, 2010
Primer2	GTGATCGGAATGGCTTGAAA	CAGCGAGCACCTGAAAGAA	Spencer and Woolnough, 2010
Primer3	ATCAAGTTTGAGGTGCTTTCC	CCATGGCATTGTGTTGAAGAC	Spencer and Woolnough, 2010
Primer4	ATTTTGCAATTTGTTCGTTCTTTC	GGAGTTTATTTGCTTCCAACACTT	Spencer and Woolnough, 2010
Primer5	TTTATAGTCAGAGAGAGTGCTG	TGTAGGGTTCATTGTAACA	Spencer and Woolnough, 2010
Primer6	AGACGGTTGGGAAGGTGGTA	CGACAGCAAGGCACAGGA	Spencer and Woolnough, 2010
Primer7	GAAGAGGTTGGGGCACTAC	CAGGCAGATATCCATTGAA	Ould Ahmed and et al. 2010
Primer8	AGACGGTTGGGAAGGTGGTA	CGACAGCAAGGCACAGGA	Ould Ahmed and et al. 2010

Genotyping was performed using geneMapper (version 3.7) software. Input files for further analyses were prepared using GenAlEx 6.4 (Peakall and Smouse, 2006). Genetic variation for each locus and populations like allele frequencies, observed number of alleles, effective number of allele (Ne), observed heterozygosity (Ho) and expected heterozygosity (He) was measured in all samples by GenAlex (6.4).

Result and discussion

All the loci were polymorphic in each population and were successfully amplified. The estimation of various genetic parameters for three populations has been given in Table 2.

Table 2: Genetic variation parameters estimated in Iranian camels

Pop	Na	Ne	I	Ho	He	UHe	F
Yazd	7.111	6.130	2.101	0.785	0.855	0.869	0.190
Semnan	6.667	5.235	1.965	0.824	0.833	0.869	0.103
Shahrod	7.444	6.970	2.090	0.771	0.850	0.862	0.202

Na: mean number allele, Ne: effective number of allele, I fixation index, Ho: observed heterozygosity, He: expected heterozygosity, UHe: unbiased expected heterozygosity and F: inbreeding coeficiemt

All loci produced 85 alleles. The mean number of alleles per locus was 5.55, 6.16, and 4.87 for yazd, semnan and shahrod populations respectively. The mean observed heterozygosity was 0.785, 0.524, and 0.771 for yazd, semnan and shahrod populations, respectively.

The results presented in this paper are the first to report on the genetic structure of the Iranian dromedary population. The degree of polymorphism revealed by the eight microsatellite markers provides important information on the genetic structure of camels in the Iran.

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Mastitis control program in a large scale camel dairy

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Introduction

Mastitis is the inflammatory response of the udder to infectious or non infectious origins and it is one of the most important diseases of lactating animals in cows, sheep, goats and camels as well. In the bovine dairy industry there is a vast amount of information, knowledge, research and experience about the etiology, epidemiology, pathogenesis, treatment, prevention and control of mastitis but it still remains a challenge for farmers and veterinarians (Bradley, 2002; Smith, 2005; Zecconi, 2005). Camels are important milk producing animals in arid and semi-arid countries. The majority of camel milk is produced by pastoral people or in traditional farming systems but the quality of the raw milk, the animal and udder health status is not monitored regularly. Data on mastitis, udder health and milk quality of camels are available only in limited numbers. Mastitis can cause significant economic losses which main elements are: decreased milk production, discarded milk, medicine cost, altered milk quality, labour, culling and spreading pathogen microorganisms. Besides the financial losses mastitis has an important animal welfare aspect as well, because it can be painful and sometimes long lasting or a life threatening disease. The Farm Animal Welfare Council (FAWC) has defined the five freedoms of animals: 1). freedom from hunger and thirst; 2). freedom from discomfort; 3). freedom from pain, injury or disease; 4). freedom to express normal behavior; 5). freedom from fear and distress.

Controlling mastitis and udder health requires a complex management system, which complies with animal health, animal welfare and consumer safety aspects and regulations. A large scale camel dairy has been established in Dubai in 2006, where the camels are milked in a herringbone milking parlour with machine. Currently there are more than 700 lactating camels on the farm. In this paper the authors would like to summarize their experience on mastitis and udder health in lactating dromedary camels.

Mastitis and subclinical mastitis

Camels have four udder quarters with two, occasionally three teat canals in each quarter. There is a connective tissue septum between the right and the left side of the udder but there is no physical separation between the front and hind quarters, although the milk ducts are not connected. Mastitis is the inflammation of the mammary gland and can be clinical and subclinical. In clinical mastitis there is a visible sign of inflammation in the involved quarters or the whole udder, such as oedema, hot, painful quarters, sometimes fever, and reluctance to move. The appearance of the milk is not altered most of the time but it can be creamy, bloody and occasionally flaky. The number of clinical mastitis cases on the farm is between 3–6 % of lactating camels. In subclinical mastitis there is no visible sign of inflammation, but there is an increase in somatic cell count, obligate pathogen bacteria is present in the milk sample and there is a decrease in milk production. Somatic cell count thresholds, neither bulk tank nor individual are not well established yet in camels, large number of data collection and analysis is going on recently on our camel dairy.

Mastitis causing agents and factors

Infectious microorganisms

Results of regular screening samples and milk samples from clinical mastitis camels (n=36083 samples), between 2006-2011 are summarized in Table 1.

Table 1: Distribution of isolated bacteria from mastitis camels and regular screening samples

Bacteria isolated	**Mastitis (%)**	**Screening (%)**
Coagulase negative Staphylococci	66.4	80.2
Corynebacterium sp/amycolatum	12.2	8.7
Staphylococcus aureus	6.2	5.9
Streptococcus agalactiae	6.2	1.1
α-hemolytic Streptococci	5.1	1.9
Str.equi ssp.zooepidemicus	1.7	0.3
Streptococcus bovis	0.7	0.5
E.coli	0.1	0.1
Others	1.4	1.3

Staphylococcus aureus, Streptococcus agalactiae and coagulase negative Staphylococci seemed to be important udder pathogens in camels according to Abdurahman (2006). Saleh and Faye (2011) found mainly Streptococcus spp., Staphylococcus aureus, other Staphylococcus and E.coli bacterial species in quarter milk samples. According to our experience the dominant bacterial flora both in mastitis and in normal screening samples as well is coagulase negative Staphylococci, mainly Staphylococcus epidermidis. This result is similar to the findings of milk bacteriological results of small ruminants (Contreras et al. 2005). Streptococcus agalactiae is a major udder pathogen, but Staphylococcus aureus alone rarely seems to be a clinical mastitis causing agent so far. E. coli causes mastitis but only occasionally. These cases are very acute and very severe, characterized by large, hard, very painful udder, fever, lethargy and may end up with one or two quarter necrosis or death of the camel. The presence of bacteria in the milk sample does not mean that the camel has clinical or subclinical mastitis, many times no elevated somatic cell count or high california mastitis test score accompanying these findings. It seems that there is a very fragile equilibrium in the udder bacterial flora. Many factors can influence this status, like udder immune system, general health of the animal, milking and the environment. Changes in these factors can disturb the bacterial flora as well and predispose the udder to clinical and subclinical mastitis.

Non infectious agents

Injury on the udder or teats can cause mastitis. Female camels sometimes fight within the group and bite the udder, the flank and perineal regions of each other.

New camels sometimes develop chronic or reoccurring mastitis shortly after they arrive to the farm. In these cases even with repeated microbiological tests no bacteriological infection can be detected. We assume that all the changes, new location, new feed, new animals in the group, less free walking and machine milking are too big stress for them and they can't adopt to the new environment as quickly as the other camels do. Many of these animals have no mastitis at all in their second lactation.

Management system to control udder health and mastitis

Monitoring bulk milk quality

After the morning and evening milking bulk milk samples are taken and checked for total viable count (TVC), coliform count, somatic cell count (SCC) and california mastitis test (CMT). The average TVC is 5413 ± 128 cfu/ml and the average SCC is 405227 ± 3537 cells/ml (Nagy at al., 2012).

Identifying and treating mastitis camels

Identifying mastitis camel is easy because of the obvious clinical signs. Quarter milk sample is taken for bacteriological examination and CMT and the animal is treated. Mastitis camels are not separated from the group, because any change within the paddock, e.g. adding or removing camels disturbs them and results in drop of milk production for couple of days. There is a proper identification and marking of the sick animal and she is milked separately during the treatment and the withdrawal period. None of the medicine available for mastitis treatment is registered for camels so we follow the EU regulation (Art. 10 of Directive 2001/82) for off labor drug use and withdrawal time.

Regular sampling of lactating camels

Milk sample is taken from every lactating camel once a month for bacteriological examination, CMT and recently somatic cell count determination. Camels with high total viable count or with secreted udder pathogens (Streptococcus agalactiae, Streptococcus zooepidemicus) are treated. With this treatment we can prevent clinical mastitis and spreading pathogen bacteria to other camels.

Milking hygiene and milking machine

Before milking the udder is cleaned with disposable udder tissue. Besides cleaning, this acts as stimulation to the milk let down reflex as well. After milking teat dip is not used. The machine milking system is thoroughly serviced and maintained twice a year, liners are changed regularly once a year.

Paddocks and exercise of the camels

Camels are kept in open paddocks with a shade in them. Camels dry off if their calves die or weaned, so we keep the camels and their calves close to each other until they reach 1 year of age. Calves are kept in paddocks adjacent to their mothers' and after each milking they are allowed to suckle the small amount of residue milk from the udder. After the morning milking the camels and calves are going to the walking track for one hour long walk.

Culling and drying off animals

Camels with chronic or more than five times reoccurring mastitis are culled. Camels with repeatedly high individual total viable count or those who secreting udder pathogens despite treatment but have no clinical mastitis are dried off.

Conclusion

Camels can be kept and machine milked in large scale, intense system. With a complex management system the occurrence of clinical mastitis can be kept between 3-6 % of lactating animals. In the future we would like to put more emphasis on individual somatic cell count monitoring and define the subclinical mastitis in camels more precisely.

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Nutritional strategies for improving growth, reproduction and milk production of dromedary camels in changing climate

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Introduction

Camelids are the most ignored domestic ruminants in the world. They are important sources of milk and meat as well as a means of transport mainly in the arid and semi-arid rangelands. The far reaching impact of natural disasters has forced many camel herders to start settling near cities, (Abbas and Omer, 2005) nearer civilization. Also the impact of climate change and peri-urban camel production has forced stall feeding of dromedary camels, although this has taken advantage of the recent advances of animal nutrition. The influence of nutrition on milk, growth and reproduction has been extensively investigated in conventional farming systems and it is concluded that decreased nutrient intake reduces the growth of calves, lowers milk production, delays the onset of puberty in heifers and increases the post partum interval to conception in female camels. This paper summarizes the nutritional strategies and technological interventions to improve the growth, reproductive and productive performance of dromedary camels.

Nutrient requirements of dromedary camels

Camels generally consume 1.0-2.5 kg dry matter/100 kg body weight depending on their physiological status (pregnancy, growth, breeding, lactation or working) and quality of feed such as its energy and protein content, stage of maturity, physical form and processing method.

Growth and maintenance

The dry matter (DM, kg), digestible crude protein (DCP, g), total digestible nutrients (TDN, kg), calcium (Ca, g) and Phosphorus (P, g) requirements of camel calves weighing 200 kg body weight are 5.0, 250, 2.0, 80 and 30, respectively for a growth rate of 100 g/day. However, for higher growth rates (300 g/day), the DM (kg), DCP (g) and TDN (kg)

requirements of camel calves (200 kg BW) are 5.0, 242 and 2.15, respectively. Rations containing approximately 4% DCP, 45% TDN, 2% Ca and 1% phosphorus should meet the requirements of adult camels, if fed as per dry matter requirement per day.

Reproduction, lactation and work

Animals in advanced stages of pregnancy, lactating or carrying out 6-8 hours of work should be provided with 25% more nutrients to meet the nutrient demand of a growing foetus, milk synthesis and work. The maintenance requirement of a 400 kg breeding female has been estimated to be 45 MJ metabolizable energy (ME) and 260 g DCP, however for production of 1 liter of milk, the additional requirement is 5 MJ ME and 50 g DCP. Lactating camels consume higher DM (58%), CP (50%) and ME (40%) than dry camels.

Interaction among nutrition-reproduction and genotype

The process of maximizing milk yield to the extent of genetic merit has been paralleled by a decrease in reproductive performance and a significantly higher incidence of metabolic and infectious diseases during the early post partum period; disappointing fertility results are a worldwide concern in the modern dairy industry. Dairy animals lose up to 10% of their body weight during the first week post partum and it has been shown that the degree and duration of energy deficit during this early post partum period is positively correlated with the number of days to first estrus. This needs an interdisciplinary approach involving animal nutrition and reproductive physiology to improve results.

Impact of climate change on dromedary camel production

Climate affects animal production in four ways: (a) impacts changes in livestock feed-grain availability and price; (b) impacts livestock pastures and forage crop production and quality; (c) changes the distribution of livestock diseases and pests; and (d) the direct effects of weather and extreme events affect animal health, growth and reproduction. Heat stress has a variety of detrimental effects on livestock, with significant effects on milk production and reproduction. Three basic management schemes for reducing the effect of thermal stress have been identified as (a) physical modification of the environment; (b) genetic development of less sensitive breeds and (c) improved nutritional management schemes.

Nutritional strategies for improving growth, reproduction and milk production

Urea-molasses mineral blocks/Multi-nutrient feed blocks

Supplementation of nitrogen, easily fermentable energy and minerals together in a block form, such as a urea-molasses mineral (UMMB) lick, improves fermentation and utilization of organic matter. The main aim is to provide degradable nitrogen in the rumen with a compatible energy source; and minerals as well as rumen non-degradable protein and medications can also be added. Supplementation of low quality feed materials with UMMB blocks showed significant impact on increasing milk production and reproductive performance in small-holder dairying in Africa and Asia.

Densified complete feed block

Complete feed blocks allow all the ingredients i.e., dry roughages, concentrates, mineral and vitamin supplements and other feed additives to get sufficiently blended so that the concentrate particulate matter sticks to the fibre particles with the help of a specific binder or molasses. Feeding of densified feed block, which is a Total Mixed Ration (TMR), increases the feed intake, meets all the nutritional requirement of the camels, and thereby improves the productive and reproductive performance. There is also a reduction in the age of first calving, persistency in the lactation curve and improved health status in camels fed feed blocks.

Feed additives

Feed additives are used to increase the health status, fertility and performance of animals as they improve the feed efficiency, mainly by increasing the digestibility of nutrients. These additives include antibiotics, organic acids, direct fed microbials (probiotics), prebiotics, enzymes and plant extracts. Fibrolytic enzymes like cellulase and xylanase increase the growth rate of calves and the performance of lactating camels. Recently, some non-ionic-surfactants (NIS) have also been reported to increase the productivity of dairy animals in terms of both growth rate and milk production.

Area specific mineral mixtures and Chelated minerals

Minerals are essential for all organisms. They contribute to the bone structure, the electrolytic balance, the protein structure, nervous and muscle activities as well as enzyme and vitamin activities. Supplementation of area specific mineral mixtures yielded a positive response in dairy animals in terms of improvement in reproductive efficiency with better productive performance i.e., increased milk yield and sustained peak milk production. In

chelated form, the trace minerals like Zn, Cu, Mn and Co are bound to some inorganic or organic ligand and thus have better absorbability in the intestine and higher bio-availability at the tissue level.

Novel feeds/Non-conventional feed resources

Feeds that do not form part of the normal diet of animals are generally termed as novel/non-conventional feeds. Many of these non-conventional feed resources result from the industrial processing of agricultural products. They are generally rich in protein and minerals and have less fiber but contain more fermentable energy than crop residues. Many of them contain toxic components or anti-nutritional factors which limit their use as animal feed beyond certain levels. Such feed resources can be classified according to their origin into the following four main types viz., feeds from field and plantation crops, feeds from tree crops, feeds from food processing industries (fruits, vegetables, human food and alcohol) and feeds from miscellaneous sources (plants, animals).

Production of designer camel milk for human health (biosynthesis of CLA)

Conjugated linoleic acid (CLA) has been shown to decrease the risk of breast cancer, increase metabolic rate, decrease body fat, enhance muscle growth, promote a healthy heart (lowers cholesterol and triglycerides), lower insulin resistance and enhance the immune system. The most predominant form of CLA, cis 9, trans-11 C 18:2, is formed in the rumen as an intermediate product during the biohydrogenation of linoleic acid by the bacterium *Butyrivibrio fibrisolvens* and other bacterial species (Harefoot and Hazlewood, 1988). The average CLA content of milk varies between 3-5.5 mg/g fat, but typical consumption of CLA by humans is only 25% of the effective dose which has been shown to reduce tumors in animal models (Ip et al., 1994). The best way to improve this is by manipulation of the camel's diet. Cows grazing on pastures containing perennial ryegrass had significantly higher CLA in their milk as compared with cows fed grass (23.0 vs 14.2 mg/g fat). It has been reported that feeding of plant oils such as sunflower, soybean, peanut and linseed increased the CLA content of milk, and feeding full fat extruded soybeans and cottonseed to dairy cows doubled the CLA content of milk and cheese (Dhiman et al., 1997). Feeding of mustard cake/mustard oil has also been reported to increase the CLA content of milk as compared with groundnut cake in milking animals. Therefore, more feeding practices need to be explored to have CLA enriched milk.

Feeding strategies to mitigate green house gases from dromedary camels

Much of the world's livestock are ruminants such as sheep, goats, camel, cattle, and buffalo-which have a unique, four/three chambered stomach. In the chamber called the rumen, bacteria break down food and generate methane as a by-product. The production rate is affected by factors such as quantity and quality of feed, body weight, age and exercise, and varies among animal species as well as among individuals of the same species. In general, 8–12% of dietary energy is lost in the form of methane in ruminants depending upon the quality and quantity of the ration. The mitigation strategies include nutrient supplementation, feeding of greens and legumes, supplementation of fat/fatty acids, microbial interventions, essential oils etc.

Conclusions

The present status of camel production in relation to the existing feed resources and the appropriate feeding strategies to improve growth, reproduction and milk production of dromedary camels in a changing climate have been discussed. The scope for the development of nutritional based interventions through bio-technological approaches has been highlighted. In addition, the future roles of interactions that exist between nutrition, production and reproduction have been emphasized to safeguard camel production from the existing poor fodder base so that the genetic potential of germ plasm can be exploited through scientific feeding.

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Effect of parasite management practices on fiber growth and quality in alpacas in the U.S. Mid-Atlantic region

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Introduction

Alpaca fiber production is a more recent agricultural activity in the U.S., but the national herd now exceeds 165,000 registered animals on 15,000 farms (Alpaca Registry, 2012). There is a range of climatic zones in the U.S., all different from the indigenous environment of the alpaca, and research-based recommendations on management practices for efficient fiber production in the various zones are lacking. The characteristics of alpaca fleeces in the U.S. have been reported by Lupton et al. (2006).

Environmental conditions in the Mid-Atlantic region make gastrointestinal nematodes a major constraint for small ruminant production. While the benefit of controlling internal and external parasites on fiber yield has been demonstrated in native South American alpaca herds (Windsor et al., 1992), little is still known about the pharmacokinetics (Jarvinen et al., 2002) and dosage of anthelmintics (Geurden and Van Hemelrijk, 2005) in camelids. The effect on parasite burden in alpacas grazing pasture contaminated by lambs has been documented in New Zealand (Hill et al., 1993), and data suggested that alpacas were less affected with gastrointestinal nematodes than sheep. There is widely documented resistance of parasites to commercial de-wormers in sheep and goats (Zajac and Gipson, 2000), and the presence of resistance to two major classes of anthelmintics on llama and alpaca farms in Georgia has been reported (Gillespie et al., 2010).

Limited information is available in the U.S. on the effect of gastrointestinal nematodes on alpaca fiber production especially in the hot and humid conditions in the south eastern U.S. This experiment evaluated the effect of deworming practices on fiber production in alpacas in this region, comparing a standard deworming program at regular intervals

largely to control meningeal worm (*Paralaphostrongylustenius*), to an 'on-demand' system of deworming individual animals when strongylid egg counts exceeded a pre-set limit.

Materials and Methods

The mature male alpacas for this research were donated to Virginia State University by regional alpaca breeders, and the research was approved by the Agricultural Animal Care and Use Committee. The 16 alpacas, following a period of quarantine and adaptation, were allocated to 2 groups of 8, blocked by body weight and fiber diameter, in March 2008. In Group 1 ('timed'), all alpacas were treated with ivermectin (0.4 mg/kg body weight; sc) at 6-week intervals following typical industry practices in this region, whereas in Group 2 ('on-demand') alpacas were treated individually with moxidectin (0.2 mg/kg body weight; oral) when strongylid fecal egg counts exceeded 200 eggs/g. Alpacas were maintained on naturally parasite-infected pastures (1 ha) previously grazed by sheep and goats. Fecal and blood samples were collected and body weight recorded at 14-d intervals throughout the grazing season. Fecal egg counts were determined using a modified McMaster technique (Zajac and Conboy, 2006) and blood samples were used to determine packed cell volume (PCV). Alpacas were shorn in May 2008, and each fleece was separated into 5 regions and each weighed before fiber analysis. Fiber characteristics were determined using standard methods including the OFDA 2000 instrument for fiber diameter-related properties (Lupton et al. 2006).

In the first production cycle, groups grazed in adjacent, but separate pastures. In November the two groups were combined in a single pasture, and supplemented with medium quality hay until pasture growth resumed. Alpacas grazed as a single herd during the second production cycle, but remained on their designated parasite management. Alpacas were again shorn in May 2009. Seven male yearling Spanish and Myotonic tracer goats were placed with the alpacas in February 2009 after treatment with a cocktail of ivermectin (0.4 mg/kg) and levamisole (11 mg/kg) to remove gastrointestinal parasites. Fecal samples were collected from the goats at the same 14-d interval and used to estimate parasite contamination of the pasture, and determine differences in fecal egg counts between the co-grazed species. Data were analyzed for the effect of parasite management on fleece characteristics separately for the two production cycles using the GLM procedure of SAS (ver. 9.1).

Results and Discussion

Alpaca body weight was not different (P>0.1) between the management groups in the first (mean: 68 kg) or second cycle (mean: 71 kg), and did not fluctuate significantly except in response to shearing. Mean fecal egg counts remained low in both groups, and only exceeded 200 eggs/g in one animal in the individually treated group during the first grazing cycle and twice in the same animal in the timed group in the second cycle. There was no effect (P>0.1) of parasite management on PCV, and no fluctuations in fecal egg counts or PCV with changing seasons in the alpacas. In contrast, mean fecal egg counts in the tracer goats increased from 0 eggs/g at introduction to pasture to a high of 1,800 eggs/g by July (Figure 1).

Figure 1: Changes in strongylid egg counts in co-grazed alpacas and goats (cycle 2)

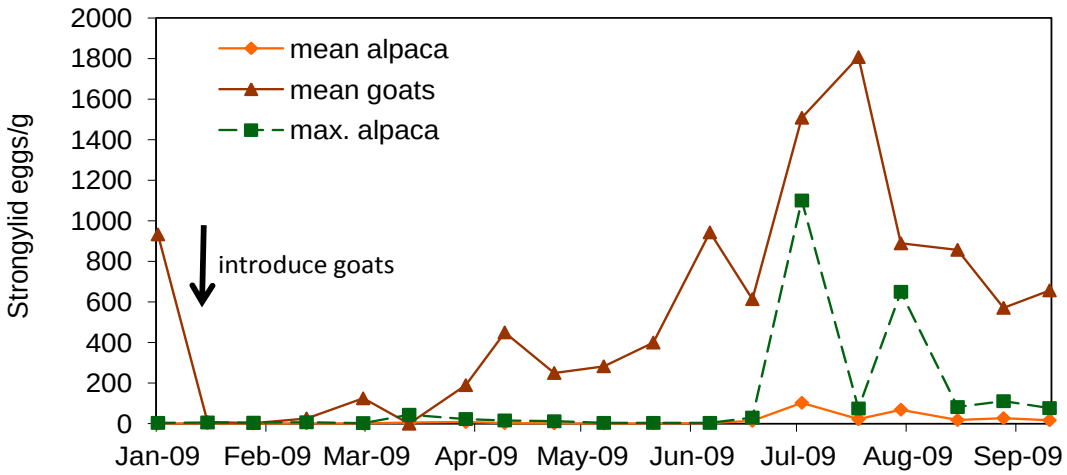

Table 1: Selected fleece characteristics of alpacas treated with ivermectin at 6-week intervals (timed) or treated individually when fecal egg counts >200 eggs/g (on-demand)

	cycle 1†		cycle 2†	
	timed	on-demand	Timed	on-demand
Clean fiber weight, kg	2.73	2.56	2.35	2.16
Clean scoured yield, %	89.6	85.2	87.1	87.8
Staple length (saddle), mm	99	94	87	83
Mean fiber diameter (saddle), µm	33.6	33.7	31.8	31.1
Comfort factor (saddle), %	39.2	40.1	48.0	53.1

†differences between treatment groups within cycle were not significant (P>0.1)

Parasite management practices had no effect on fiber growth (Table 1). Fleece weight was not different (P>0.1) between groups in cycle 1 (mean: 2.64 kg) and 2 (mean: 2.26 kg), nor were staple length (cycle 1: 98 mm; cycle 2: 85 mm), or fiber diameter (cycle 1: 33.7 µm; cycle 2: 31.5 µm). There was also no difference in fiber quality characteristics (i.e. staple strength, comfort factor, medullation).

The experiment showed lower gastrointestinal nematode infection in alpacas compared to goats, in line with observations by Hill et al. (1993) when co-grazing alpacas with lambs. The level of gastrointestinal nematode infection resulting in clinical symptoms is not well documented, however, packed cell volume remained constant and within normal limits in this experiment. Although the effect of routine deworming was masked by the presence of anthelmintic resistance to ivermectin in gastrointestinal nematodes at this location, results indicate that limiting deworming to animals with increased fecal egg counts did not affect body weight, and fiber production and quality. Reducing the use of dewormers may be helpful in delaying the further development of anthelmintic resistance.

Acknowledgements

This research was funded by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Capacity Building Grant ProgramAward No. 2008-38814-04730

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Improving breeding success for alpaca farmers in the southern Peruvian Highlands

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This abstract describes the start of a breeding improvement program in the Nunoa District of the Puno Region in the southern Peruvian highlands. The area depends primarily on alpaca wool production for its livelihood. Four communities are under investigation. All have been in existence for several years and produce alpacas, llamas, sheep, and cattle. Different degrees of breeding success were discovered on the farms. One of the communities is very successful with many animals with excellent wool, and pregnancy and alpaca birthing rates of 70 to 80% verified by ultrasound examination. It has achieved this success with careful management and outside financial support. The other three communities have no outside financial support as is typical for most farms in the area, and they report birthing rates of between 30 and 50%. The low birthing rates are due to too few quality breeding males and too high a female to male ratio. The wool of many of the animals on these farms is poor in quality and quantity. The most successful farm has excellent production records. Two of the other farms have minimal records and the third has no record keeping system. It is the least productive farm in terms of birthing rates and wool production. A breeding improvement program was undertaken in January 2012 by a US non-profit organization (Nunoa Project, www.nunoaproject.org) with veterinary support from US and Argentinean professionals. Six top quality males were purchased by the Nunoa Project from the top producing community herd and two were given to each of the other three communities selected by the local municipality's animal health and production authorities. These communities are not financially able to purchase new quality males and thus cannot break the cycle of poor production. The concept is to use the offspring of the Nunoa Project males to produce an elite

breeding herd to improve the production of the community herds over time. The males are to be rotated among the 3 communities after 2 years.

The first investigations of alpaca farms in Nunoa District were undertaken by members of the Nunoa Project in 2009. Community A consists of 4 families and approximately 27 members, and is the one with the highest production of offspring and quality wool. These families care for approximately 800 alpacas. They do not produce any plant crops like the other three communities do. They use 10 males to breed their 350 reproduction females. Their pregnancy rate has been validated with ultrasound at 70 to 80%. The Peruvian breeding season runs from December until March, and teams from the Nunoa Project performed all pregnancy checks in August in 2010 and 2011. The quality of the males is very good in this community as they started with foreign financial backing and have a very progressive herd manager. He is able to purchase top quality males from a large cooperative farm in the area. He uses production records to make informed decisions about breeding females and removes those with poor production. If a female does not produce an offspring after 3 years of 90 day exposure to breeding males, then she is no longer exposed to males and joins the non-breeding, wool production only herd. If the males assigned to a breeding group do not achieve a high pregnancy rate in one year then they are replaced with other quality males from the herd or other males purchased from outside the herd. Semen analysis was performed by Nunoa Project veterinarians in 2011 and 2012 on a small number of males at this farm. Samples were obtained from a postcoital vaginal collection. This is not performed on a widespread basis in Peru but it would help to identify low fertility males and poor breeding practices. A field study of males is planned for the 2012-2013 breeding season at Community A. The goals are to analyze semen from males during the breeding season and to teach the herd manager to perform ongoing analyses. He in turn can teach farmers from other communities so that male production can be monitored and corrections made early if problems are discovered.

Community B consists of 20 families and approximately 110 members. They have approximately 600 alpacas and breed 300 females per year. Families own their own animals and there is also a community alpaca herd. In January 2012 the Nunoa Project assisted the community in selecting the community herd females to be pastured with the two new Nunoa Project males. The starting group consisted of approximately 50 females, some nursing young crias, and the group had been pastured continuously with two adult males. The community members checked all animals for pregnancy by abdominal palpation, and those that were not found to be in advanced pregnancy were separated out and examined. All animals were

checked for age and body condition. One was rejected because of extremely poor body condition and a purulent vaginal discharge. The 25 females selected were identified with new ear tags. The two new males were to be pastured with the selected female group for 90 days. In July of 2012 the females are to be checked for pregnancy by ultrasound. Some females will of course have been bred by the two males with which they had been pastured prior to the introduction of the new Nunoa Project males.

Community C consists of 23 families and approximately 130 members. They have approximately 400 alpacas and breed 260 females per year with 10 males. In January 2012 they selected 30 females to breed with the two new Nunoa Project males which are kept in a separate location. They planned to manage them by exposing 5 females to the 2 males daily and allowing breedings to take place if females were sexually receptive. They have ear tags on the females and keep records of the breedings. In July of 2012 the females will be checked for pregnancy by ultrasound.

Community D consists of approximately 100 members raising 1800 alpacas. A total of 800 females are exposed to 8 males per year. The ratio of 100 to 1 is an extremely high female to male ratio which usually results in low pregnancy rates. They are at a disadvantage with respect to the other two communities because they do not have fencing to separate breeding males from the rest of the herd. The animals do not have ear tags and no breeding records are kept because the community members are not literate. The local municipality agreed to provide ear tags to the herd and also to help them establish a record keeping system so that they can identify and cull non-productive animals and thus help to improve production. The two Nunoa Project males were added to the rest of the herd. This method of breeding will not result in improvement to the breeding herd as rapidly as establishing a limited breeding group as in the other two communities. The addition of the new genetics to the community herd will help improve wool production and birthing rates over time.

Ongoing local municipality and Nunoa Project support is planned to improve alpaca production. This will be a gradual process and includes seminars with local producers emphasizing record keeping and careful selection and evaluation of breeding males and females.

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Production and reproduction performance of camel herds under different production systems in Egypt

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Introduction

The camel is the best provider of meat and milk in arid areas (Sibtain et al., 2012). It plays an important role in livestock production in Egypt, especially for the pastoral communities and is an important source of meat for arid and breeder provinces. A rapidly increasing population has directed attention to meat production from this marginalized animal; the meat is either produced locally or imported from neighboring countries.

The low reproductive performance of camel herds is a primary factor affecting their productivity, and can be caused by late puberty and long calving intervals (Kaufmann 2005). The objectives of this study were to assess the production and reproductive performance of different breeds of camels raised under different agro-ecologies and production systems in Egypt.

Materials and methods

Seventy-seven breeding herds in four camel production regions in Egypt were studied during 2010/2011. The four regions were the North Coastal Zone of the Western Desert (Matrouh governorate) characterized by a pastoral production system; the South border with Sudan (Aswan governorate), with an intensive crop-livestock production system; the East Delta region (Sharkia governorate), with a small scale house-hold production system and the desert Oasis region (New Valley governorate) with mixed production system (Aboul-Naga et al. 2012). The numbers of breeding herds in the studied locations were 29, 6, 11 and 27 respectively. The data were collected through field visits to the breeding herds using a pre-designed questionnaire. The questionnaire included information on camel breed, mating season, conception, calving and weaning, birth and weaning weights, daily milk yield, and calves fattening age and weight at marketing.

Results and Discussion

Mating systems differ significantly among the studied agro-ecological regions. The sire was present in the herd only during the breeding season (November - March) for most of the herds at Matrouh and Sharkia (93% and 82% respectively), whilst the sire was with the herds all year round in 50% of Aswan and 63% of New Valley herds.

Age at first mating was 3.9 years, which was significantly higher (P<005) in Maghrabi camels under the pastoral system in Matrouh than in the other regions (Table 1). Females of the same breed were first mated at 3.2 years in the New Valley where green fodder was available. On the other hand, the mating season was significantly shorter in Matrouh (3.2 months) than in the other three locations, and the number of services required for conception was significantly (P<0.05) lower than in Sharkia and New Valley. The reproductive efficiency of camels under the harsh natural conditions was relatively low, which is in agreement with the findings of Skidmore (2003).

Table 1: Reproduction performance in the studied camel production systems

Reproduction traits	**Matrouh (Pastoral) Mean ±SE**	**Aswan (Crop-livestock) Mean ±SE**	**Sharkia (Household) Mean ±SE**	**New valley (Desert oasis) Mean ±SE**
No. of breeding herds	29	6	11	27
Age at first mating (yrs)	3.9 ± 0.13 [a]	3.5 ± 0 [b]	3.2 ± 0.10 [b]	3.2 ± 0.07 [b]
Length of mating season (mo.)	3.2 ± 0.19 [a]	5.5± 0.34 [b]	5.1 ± 0.16 [b]	5.1 ± 0.20 [b]
No. of services/conception	1.54 ± 0.12 [a]	1.30 ± 0.21 [a]	1.73 ± 0.19 [b]	1.97 ± 0.06 [b]
No. of females/sire	35.6 ± 2.31 [a]	35.7 ± 13.4 [a]	56.8 ± 6.72 [b]	53.3 ± 3.08 [b]
No. of females mated	35.2 ± 6.46 [b]	6.2 ± 3.53 [a]	13.1 ± 1.64 [a]	11.9 ± 3.88 [a]
No. of females calved	25.7 ± 4.19 [a]	6.0 ± 3.52 [b]	12.7 ± 1.63 [ab]	10.3 ± 3.36 [a]
Conception rate	0.80 ± 0.05 [a]	0.98 ± 0.02 [b]	0.97 ± 0.02 [b]	0.88 ± 0.04 [a]
No. of calves/ female mated	25.3 ± 4.28 [a]	5.7 ± 3.2 [b]	12.2 ± 1.53 [ab]	10.2 ± 3.29 [b]
Mortality before weaning (%)	4.8 ± 0	1.0 ± 0	1.0 ± 0	1.9 ± 0.26
Mortality after weaning (%)	1.0 ± 0	0	1.3 ± 0.33	2.0 ± 0

* Means followed by the same letter within each trait, did not differ significantly (P<0.05) according to Duncan test.

The conception rate was significantly higher (P<0.05) for camel herds raised under the crop-livestock production system in Aswan and Sharkia, than for herds raised under the pastoral system (98, 97 and 80%, respectively). This result indicates the significant positive effect of the availability of feed resources (intensive crop- livestock system) on camel reproduction. Furthermore, the Sudani camel seems to have a higher reproductive performance than the Maghrabi camel. However the breed difference cannot be tested with our data as production system and breeds are confounded: the Sudani camel is the main breed raised in Sharkia and the only one in Aswan, while the Maghrabi camel is the main breed in The New Valley and the only one in Matrouh. Mortality up to weaning was also affected by the production system, it was significantly higher (P<0.01) under the pastoral system in Matrouh (4.8 %) compared with the other studied locations (< 1.9 %). Calf birth weight also differed significantly (P<0.001) between the production systems. Maghrabi camels raised under pastoral conditions in Matrouh and the New Valley produced lighter calves at birth (24 and 26 kg, respectively); whereas, Sudani camels under the crop-livestock system in Aswan and Sharkia produced heavier calves (36 and 31 kg, respectively) (Table 2).

Table 2: Production performance in the studied camel prevalent regions

Production traits	**Matrouh (Pastoral) Mean ±SE**	**Aswan (Crop-livestock) Mean ±SE**	**Sharkia (Household) Mean ±SE**	**New valley (Desert oasis) Mean ±SE**
No. of breeding herds	29	6	11	27
Calf birth weight (kg)	23.7 ± 1.88 [a]	35.5 ± 1.61 [b]	30.9 ± 1.32 [bc]	26.5 ± 0.91 [ac]
Calf weaning weight (kg)	117.3 ± 22.16 [a]	125.0 ± 5.48 [a]	121± 7.95 [a]	110.4 ± 8.02 [b]
Marketing weight (kg)	137 ± 13.2 [a]	360 ± 34.1 [b]	185± 26.9 [a]	130 ± 11.2 [a]
Age at marketing (month)	8.7 ± 0.58 [a]	31.2 ± 2.42 [b]	14.1 ± 4.47 [c]	8.0 ± 0.79 [a]
Price/ head (L.E)	2817 ± 139	6700 ± 583	5333 ± 572	3666 ± 170
Range	(1000-4000)	(4500-8000)	(4000-7500)	(2500-5000)
Daily milk yield				
2 – 3 ltr. (%)	80	100	90	78
4 - 5 ltr. (%)	20	0	10	22

* Means followed by the same letter within each trait, did not differ significantly (P<0.05) according to Duncan test.

Calf weaning weights were also lower under the pastoral system in Matrouh and the oasis system in New Valley, compared with the crop-livestock system in Aswan and Sharkia. Aswan and Sharkia breeders are practicing fattening of young males before marketing. The fattening process is extended up to 30 months of age in Aswan and 14 months in Sharkia to reach 360 and 185 kg, respectively. While in Matrouh and New Valley breeders market their calves' directly after weaning (8 months) at on average 136 and 130 kg body weight respectively. Accordingly, the price per head differs significantly according to marketing weight (Table 2).

The herders in Aswan and Sharkia stated that daily milk yield of nearly all their camels was around 2-3 liter/day, while in Matrouh and New Valley 20% and 22% of the camels respectively, were reported to give up to 4-5 liter/day despite the limited water and feed resources. This result may also be related to breed differences as the Maghrabi camel is regionally known as a dairy camel whereas, the Sudani camel is known as a meat breed.

Milk produced from camel herds in the four regions is mainly home-consumed, except for 10 and 38 % of the herders in Sharkia and Matrouh respectively that sell camel milk at the market.

The results of this study indicate that camel herding is an important enterprise in the pastoral areas of Matrouh and New valley governorates as a low-input, low-output production system. Camel trading, with fattening of calves, is the main camel enterprise in Aswan and Sharkia utilizing the imported camels from Sudan, Eritrea and Somalia. The performance of the imported camels (Sudani) was significantly better than camels kept under the pastoral system, as they had better feed input. However, the pastoral system in Matrouh and New valley is a sustainable system under the dry conditions of the desert, where no other livestock can reproduce.

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