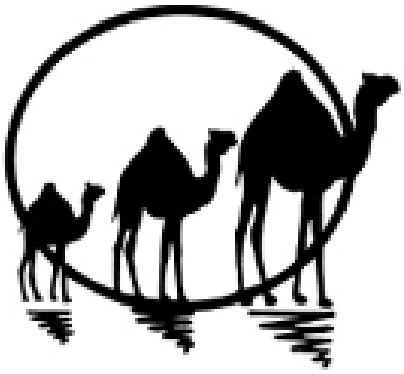

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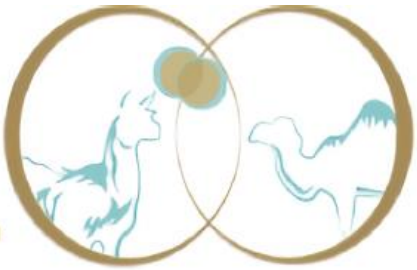

Camelid
Reproduction
1st – 3rd July

Bologna, Italy

Eds: J. Juhasz, J. Vaughan, P. Nagy, B. Mulligan, JA. Skidmore

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International Camel Organization

PROGRAMME AND EXTENDED ABSTRACTS

ICAR 2020 + 2 SATELLITE MEETING ON CAMELID REPRODUCTION

Organized by

Camel Reproduction Centre

Dubai, United Arab Emirates

and

Emirates Industry for Camel Milk and Products (Camelicious)

Dubai, United Arab Emirates

at

University of Bologna

Bologna, Italy

ICAR 2020 + 2 SATELLITE MEETING ON CAMELID REPRODUCTION

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Dr. Peter Nagy, Emirates Industry for Camel Milk and Products, Dubai, UAE
Dr. Judit Juhasz, Emirates Industry for Camel Milk and Products, Dubai, UAE
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Preface

New and Old World camelids are having an ever-increasing impact on human lives, whether it be in racing and beauty competitions, or for milk, meat, fibre, transport or tourism. Our aim is, therefore, to draw attention to this important species by gathering the scientific community and international organisations to support the development of camelid research and to enhance the transfer of knowledge from science to practice.

The 19th International Conference on Animal Reproduction (ICAR) being held in Italy this year, bringing together many international scientists and professionals, presents an ideal opportunity to organize the 4th Satellite Meeting on Camelid Reproduction at the University of Bologna.

It has not been easy this time as, originally, the meeting was planned for 2020 but had to be postponed until 2021 and then again until 2022 due to the COVID pandemic and restrictions on travel and social gatherings. I would like to thank all of you for your patience and support during this time and in particular to all the Committee members who have worked very hard promoting the meeting, finding sponsors and editing manuscripts and to Barbara Padalino and her team for all the local organization.

None of this would have been possible, however without the support of our sponsors, so particular thanks go to our: PLATINIUM SPONSOR **International Camel Organization**, SILVER SPONSOR, **IMV Technologies**, BRONZE SPONSOR **German Standard Group** and to **Animals' Angels** for sponsoring the WELFARE session, all of whom have helped make this meeting a reality.

I am delighted that this year we can go ahead with the conference and now meet face to face, which I am sure we have all missed over these last couple of years. It promises to be an exciting programme with reviews and original research papers in all aspects of camelid reproduction, together with some fun social events including a city bus tour with drinks and canapes in the park and a day trip to visit the beautiful town of Ferrara and Castle and a tour of a dairy and cheese factory which I am sure will be memorable too.

Looking forward to welcoming you to Bologna, Italy.

Dr J.A. (Lulu) Skidmore
Chair, Camelid Satellite Meeting

Scientific Programme

FRIDAY 1st July 2022

8.30 – 10.00 Registration

10.00 – 10.15 Welcome and introduction to International Camel Organisation (ICO)

SESSION 1	**Embryology Chaired by Dr. Peter Nagy**	
Time	Presenting author	Title
10.15 - 10.45	G. Vajta	Tips and tricks in vitrification of embryos
10.45 - 11.00	JA. Skidmore	Update on cryopreservation of camel embryos
11.00 - 11.15	BP. Mulligan	Expansion and pregnancy rates of grade 3 folded dromedary camel embryos, following overnight incubation at 37°C
11.15 - 11.30	Discussion	
11.30 – 12.00	**TEA/ COFFEE**	
SESSION 2	**Female Reproduction Chaired by Dr. JA (Lulu) Skidmore**	
12.00 - 12.15	BM. Manjunatha	Superovulation with eCG in dromedary camels
12.15 - 12.30	D. Monaco	Macroscopic features of dromedary camel placenta at term
12.30 - 12.45	P. Nagy	Pregnancy losses in dromedary camels
12.45 - 13.00	C. Whitehead	Evaluation of relaxin concentrations for pregnancy testing in Alpacas using a stallside test
13.00 - 13.15	Discussion	
13.15 - 14.15	**LUNCH**	
SESSION 3	**Male Reproduction Chaired by Mr. Brendan Mulligan**	
14.15 - 14.30	CM. Malo	Use of reproductive ultrasound for fertility investigations of male camels
14.30 - 14.45	N. Mansour	Semen collection from dromedary camel bulls, methodology and a new patented approach
14.45 - 15.00	A. Niasari-Naslaji	Problems and solutions associated with camel semen collection and viscosity
15.00 - 15.15	JM. Morrell	Colloid centrifugation of alpaca semen
15.15 - 15.45	**TEA/ COFFEE**	

SESSION 4	**Male reproduction Chaired by Dr. Clara Malo**	
Time	Presenting author	Title
15.45 - 16.00	EG. Aisen	Seasonal variations on morphometry and abnormalities of alpaca (*Vicugna pacos*) spermatozoa
16.00 - 16.15	W. Huanca	Use of seminal plasma to induce ovulation in alpacas (*Vicugna pacos*) to improve embryonic survival on day 5 or 7 after mating and pregnancy rates in embryo recipients
16.15 - 16.30	M. Ratto	Unveiling the luteotrophic role of beta-NGF present in the seminal plasma of llamas
16.30 - 16.45	W. Huanca	Effect of seminal plasma on the retrieval rate and quality of cumulus-oocyte complexes from ovum pick up in alpacas (*Vicugna pacos*)
16.45 - 17.00	Discussion	
18.00 - 20.00	**CITY BUS TOUR IN BOLOGNA WITH WELCOME DRINK**	

SATURDAY 2nd JULY

SESSION 5	**Nutrition, milking and camel calf Chaired by Dr. Judit Juhasz**	
Time	Presenting author	Title
9.00 - 9.15	JL. Vaughan	Selenium supplementation in camels using a depot injection of barium selenate
9.15 - 9.30	C. Castagnetti	Venous blood lactate concentrations in healthy dromedary calves at birth and at 24 h of age
9.30 - 9.45	T. Osman	Blood gas analysis in healthy dromedary calves during the first 3 weeks of age
9.45 - 10.00	M. Probo	Agreement between serum Brix refractometry and IgG, γGT and total protein in dromedary camel calves
10.00 - 10.15	M. Ayadi	Implementation of the new milk recording scheme in dairy camels
10.15 - 10.30	Discussion	
10.30 - 11.00	**TEA/ COFFEE**	
SESSION 6	**Welfare Chaired by Dr. Claire Whitehead Sponsored by ANIMALS' ANGELS**	
11.00 - 11.15	J. Juhasz	Animal health and welfare in intensive camel dairy farming
11.15 - 11.30	B. Padalino	Welfare of dromedary camels: what do we know?
11.30 - 11.45	M. Zappaterra	Camel breeding in Doha market: a survey of the caretakers

11.45 - 12.00	Discussion	
SESSION 7	**Cell culture**	**Chaired by Dr. Elena Ciani**
Time	Presenting author	Title
12.00 - 12.15	C. Iglesias Pastrana	Dromedary camel urine limits proliferation and modifies cell morphology in human renal tumoral and normal cells
12.15 - 12.30	MN. Sgobba	2-PBA, a small molecule observed in dromedary urine, induces morphological changes in secondary human renal cell lines
12.30 - 12.45	Discussion	
12.45 - 14.00	**LUNCH**	
SESSION 8	**Genetics**	**Chaired by Dr. Elena Ciani**
14.00 - 14.15	PA. Burger	New genomic tools in Old and New World camelids for assessing reproductive and other phenotypic traits
14.15 - 14.30	S. Bruno	Refining the Camelus dromedarius myostatin gene polymorphism through world-wide whole-genome sequencing
14.30 - 14.45	SY. Al Ramadan	Muc1 expression in both uterine horns of dromedary camel during peri-implantation window
14.45 - 15.00	M. Moqbel	Temporospatial expression of osteopontin in both left and right uterine horns during the peri-implantation period of Dromedary camel
15.00 - 15.15	MA. Zayed	Genetic contribution of myogenicfactor 5 and growth hormone genes for live body measurements, carcass traits and meat quality of dromedary camel
15.15 - 15.30	Discussion	
15.30 - 16.00	**TEA/ COFFEE**	
16.00 - 16.15	**Concluding remarks**	

SUNDAY 3rd JULY (9.00 am – 17.00 pm)

Field trip to Ferrara and visiting Azienda Caretti dairy and Parmigiano cheese factory.

Agricola Caretti (caseificiocaretti.it).

The organizers reserve the right to change order and content of this programme.

Table of Content

Tips and tricks in vitrification of embryos

Vajta, G

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Introduction - the phenomenon

Vitrification is probably the simplest technology in mammalian embryology, although we have to admit that none of our methods is extremely complicated. Still, we have some problems when talking about vitrification, including the very first point: definition.

Vitrification, as a physical phenomenon, is hard to define. We know well that there are four common phases of the matter, solid, liquid, gaseous and plasma. Humans live among the first three phases and experience only briefly the plasma phase at lightning or (in a most unfortunate but increasingly possible situation) at a nuclear bomb explosion. Vitrification belongs to none of these phases. It is a kind of transition between the solid and liquid, preserving the structural features of liquid but missing the most important characteristic - movement. Probably the best - or at least most plausible - definition is that vitrification is an extreme increase in the viscosity of solutions. The crucial word in that sentence is the "extreme", and the established absolute viscosity should not be mixed up with the room temperature viscosity of cryoprotectant solutions.

How does it happen? Being acknowledged as a handyman in embryology - and also according to the title of this invited talk - I am fortunately not supposed to present here curves and equations. Vitrification, glassy transition may even occur in nature, and it is not restricted to water - glass, porcelain, and cotton candy are all vitrified solutions. To induce vitrification, i.e. ice crystal-free solidification in water, requires some special conditions, including high cooling rates. There are some well-known chemicals - called cryoprotectants - that also prevent ice formation; the higher their concentration, the lower cooling rate is required. Increasing hydrostatic pressure may also facilitate vitrification, but the practical value of this approach is limited when we talk about live cell preservation. Finally, a small volume of the solution not only helps to achieve high cooling rates but prevents ice nucleation, too.

As ice formation is far the most harmful phenomenon during cryopreservation, vitrification may offer special benefits. In fact, it has been revealed that in traditional freezing, as the result of the stepwise concentration of cryoprotectants inside and around the sample, a small but crucial part of the solution also vitrifies. Strangely, initial dominant concerns regarding vitrification, i.e. the possible harmful effect of the concentrated cryoprotective additives (CPAs), were found unsupported, as the final intracellular concentration of these chemicals is higher at traditional freezing than during vitrification.

The practical procedure

In the early '90s, a robust industrial background was developed for the traditional freezing of male gametes and embryos. For an alternative procedure, a higher efficiency was not enough - the new approach had to be extremely simple, inexpensive and affordable. Although vitrification has eventually met all these criteria, it took almost three decades and a whole new generation of scientists to accept it and acknowledge as the dominant way for cryopreservation in reproductive biology.

The principal steps of this road were:

1. to increase considerably the cooling rate (i.e. >20,000ºC/min)
    - with a small volume (i.e. <1 µl), and
    - with direct immersion into liquid nitrogen (LN_2)
    - using a carrier tool to prevent floating on the surface;
2. to optimise the parallel processes of:
    - equilibration (to protect intracellular structures with CPAs), and
    - dehydration (to minimise the intracellular water content)
    - and to decrease the specific toxicity and osmotic stress during the process by stepwise addition of empirically selected permeable and impermeable CPAs.

The final procedure was established in the middle of '90s, and is used - with minimal adjustments - up till today. Although an estimated >100 versions of vitrification have been developed during the past 25 years, practically none of them was competitive with the original procedures.

However, in spite of the great achievements, we have to acknowledge that vitrification is an unphysiological and very drastic intervention. With equilibration, we poison samples with a highly concentrated toxic mixture, and the process of dehydration is comparable with a car crusher. Still, the worst part is the warming and dilution, when the already damaged structures face new challenges, toxic and structural injuries, while they are completely paralysed, no repair mechanism, no chaperons; and are expected to recover in no time to fulfil their destiny - to start a new life. I strongly suppose they deserve our special attention and care.

Suggestions for the everyday work

Choosing and learning the proper technique

Although vitrification is far the best approach for cryopreservation in embryology, it works with (close to) 100% overall efficiency only in a few species and developmental phases, including human embryos and oocytes, and *in vitro* produced bovine blastocysts. The efficient technology transfer requires personal teaching and cannot be done in a day or two. For a beginner, a few weeks in a cattle IVF laboratory - with authentic records of excellent survival and developmental rates after vitrification - may offer a unique opportunity to learn and practice a successful method.

Adapting vitrification for the given task

Establishing and stabilising the procedure in a new lab may require additional weeks, not speaking about the inevitable subsequent modifications to optimise the outcome in the given species. I suggest a systematic approach with careful small steps and very few embryos per group to minimise wasted time, material, and efforts. Fortunately, vitrification is one of the few biological procedures where the outcome is almost always obvious in a day; accordingly, hundreds of variations can be tested in a few weeks. Large-scale publishable experiments with statistically significant outcomes can be done when you already have the efficient technique.

Laboratory arrangement

Although no expensive equipment or special instruments are needed, the required simple conditions for vitrification should be taken seriously. A separate laboratory area has to be assigned for vitrification, with no air vent, no vibration, and only with tools - but all tools - needed for cooling and warming. As all steps are temperature-sensitive, heated stages, benches and precisely maintained (definitely not "ambient") degrees

(preferably 25ºC) are suggested. We should not forget that LN_2 is a highly hazardous material. Although none of the cooling-warming procedures can be performed in compliance with the standard work safety rules, our indispensable wrongdoings should not make us irresponsible above the absolutely unavoidable level. Moreover, in contrast to the painful but usually mild freezing injuries, we have to remember that LN2 vapour may kill people in unventilated storage rooms or when Dewars are transported in the closed passenger compartment of cars.

Chemicals and sources

Among the CPAs tested, the equal proportion of ethylene glycol and dimethyl-sulphoxide (DMSO) is the most popular, supplemented with a non-permeable additive. As the quality of commercially available but simple solutions may vary, I suggest an in-house prepared backup for quality control and troubleshooting. The only challenging issue is DMSO, which should be purchased/stored in tightly closed small dark bottles or ampoules and used in a few days after opening to avoid formaldehyde formation. The ready-to-use mixtures can be stored infinitely in aliquots (like all media in mammalian IVF) in a -80ºC freezer, probably the best but largely neglected instrument for an embryo laboratory (D. Brandao, *unpublished*, but confirmed by many teams).

Personal approach

We should not forget: vitrification is not a social event; it requires a calm, quiet environment that allows a strong focus on our precious samples. Both cooling and warming consist of short active steps separated by longer pauses. Still, I suggest remaining there during the whole procedure. In contrast to embryo culture, embryos need our attention and care during vitrification. We also need the information that we can collect - sometimes subconsciously - from hundreds of samples that behave variously to our sublethal insults. These seemingly dull moments may help us understand what is happening, avoid mistakes, and improve the procedures.

Closing remarks

The widespread use of vitrification is probably the most remarkable advancement in mammalian embryology during the first decades of the new millennium. Efficient cryopreservation of oocytes and embryos opened new possibilities and increased the efficiency and applicability of other techniques, too. Still, there are many things to do including adaptation for various species and developmental phases; transforming the

middle-age manual method into an automated procedure; and eliminating work-safety and disease transmission problems. Some of the most important and urgent tasks are finding an efficient way for technology transfer, avoiding regrettable differences between laboratories, and promoting the application of the most appropriate existing procedures for the given purpose.

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Update on cryopreservation of camel embryos

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Introduction

Fresh embryo transfer has been widely practiced in camelids with pregnancy rates of 65-75% routinely achieved in dromedary camels. However, the inability to cryopreserve camelid embryos to commercially acceptable levels has hindered the broader application of embryo transfer in these species. The ability to successfully cryopreserve embryos would overcome the need to transport valuable animals, reduce the need to synchronise recipients with the donor, reduce transmission of various diseases and create a means of preserving the genetics of elite, or endangered, animals long after their demise. The desire to utilise elite genetic resources from camels both nationally and internationally has led to increased interest in optimising cryopreservation techniques for camel embryos.

Optimisation of the vitrification protocol

Initial attempts to cryopreserve camel embryos using slow-cooling or vitrification methods achieved pregnancy rates of around 38%. However, as vitrification is a simpler, and more rapid method of freezing embryos, and needs less sophisticated equipment, recent studies have concentrated on improving vitrification protocols.

Several factors can affect the success of vitrification such as toxicity of cryoprotectants, embryo carrier device and embryo size. These have been investigated in recent studies in attempts to improve pregnancy rates achieved from vitrified embryos.

Toxic effect of cryoprotectants

The toxic effects of permeating cryoprotectants on embryos have been previously recognised in a variety of animal species. For example, dimethyl sulfoxide (DMSO) is used successfully in human, cattle and mouse cryopreservation but has been shown to be toxic to camel blastocysts. Herrid et al. (2016) exposed camel embryos to vitrification

solutions containing either ethylene glycol (EG) and DMSO, EG without DMSO or EG and glycerol (GLY). The viability of embryos post-thaw did not differ between groups up to 24 h after warming, but more embryos survived in the 48, 72 and 96 h culture in the EG-containing groups than in the EG and DMSO group (83%, 78% vs 59% at 96 h for EG + GLY, EG and EG + DMSO respectively). The addition of 3% GLY to the vitrification media did not improve embryo survival rate but it did appear to be less restrictive on timing, as survival rate of embryos exposed to vitrification media for any time between 40 and 60 seconds in the EG + 3% glycerol was no different from those in the EG group only, which required a rigidly controlled exposure time of 40 seconds.

Although there have been several studies on the toxic effects of permeating cryoprotectants on embryos, there have been fewer studies on the toxicity of non-permeating cryoprotectants, such as sucrose, trehalose and galactose. Herrid et al. (2016; 2017) therefore investigated different combinations of cryoprotectants and sugars for vitrification and rehydration of camel embryos. After vitrifying embryos in a combination of EG and GLY (final concentration 3.4 M GLY + 4.6 M EG) embryos were thawed and rehydrated in either 0.5 M galactose or 0.5 M sucrose solutions (0.5 M for 1 min, 0.25 M for 5 min). No differences were observed in the development rates at 48 h post thaw, 78.3% vs 85.7% for sucrose and galactose warmed embryos respectively. However, the survival and developmental rate of vitrified embryos warmed in galactose was greater than that of the sucrose group at 96 h (71.4% vs 24.0% respectively). These results indicated a slow and delayed toxic effect of sucrose and supported the observation that no pregnancies resulted from embryos that were vitrified and warmed in sucrose solutions but promising pregnancy rates of 46.1% were achieved from vitrified embryos warmed in galactose solutions. This could suggest a possible species-specific toxic effect of sucrose on camel embryos.

Studies by Herrid et al. (2016; 2017) led to the development of a vitrification kit for camel embryos (Vita Vitro, Shenzen, China) which was evaluated for use with camel embryos by Skidmore et al. (2020). As the majority of embryo vitrification protocols include supplementation of the holding media with foetal calf serum (FCS), to protect cell membranes, and bovine serum albumin (BSA), for its buffering and chelation actions, Skidmore et al, (2020) evaluated the efficacy of this kit supplemented with FCS with or without the addition of BSA. Embryos were equilibrated and vitrified in three steps, loaded into open pulled straws (OPS), and stored in liquid nitrogen. The viability after warming, and the pregnancy rates after transfer, were not different in either group with,

or without, BSA (86% vs 98% and 26% vs 48% respectively) although, the pregnancy rate in the group without BSA (48%) was numerically greater than with BSA (26%). These results indicated that the addition of BSA did not enhance embryonic re-expansion or pregnancy rates suggesting perhaps that the FCS supplemented in the kit provided adequate protective functions.

Embryo size

Previous studies have indicated that embryo size also influences the outcome of cryopreservation, whereby smaller embryos (< 500 μm) survive vitrification better than larger embryos (500-850 μm). This stage-dependent sensitivity of camel embryos is a major obstacle to a practical application of the vitrification method. The primary reason for this could be the formation of ice crystals within embryonic cells, which is associated with a slower penetration of cryoprotectants and subsequent incomplete dehydration of larger embryos. Attempts to overcome this problem have involved adopting a process of artificial shrinkage either by physically removing the blastocoels fluid via needle puncture or by incubating embryos in high osmotic solutions. Herrid et al. (2017) examined the effects of artificial shrinkage, by either puncturing the blastocoel cavity with a micro-needle or adding sucrose to the vitrification solutions when vitrifying camel embryos. The results showed that although after 48 h post-thaw survival rates were 64% and 80% for embryos that had undergone artificial shrinkage or had sucrose added to the vitrification solutions respectively, no pregnancies developed in either group. This indicated that neither artificial shrinkage nor addition of sucrose to the vitrification media improved the outcome of vitrification.

Embryo storage devices

The embryo storage device can also affect the outcome of vitrification. With the advent of minimum volume cooling, the development of different embryo storage devices has occurred, such as the Cryolock® (Irvine Scientific, USA) and Fibreplug™ (Cryologic, Australia) in addition to the OPS. Skidmore et al. (2021), compared the Cryologic Vitrification Method™ (CVM; Cryologic, Australia), whereby the embryo is vitrified in a droplet of media on the hook end of a Fibreplug™ by touching a chilled aluminium block cooled in liquid nitrogen, with the Cryolock® method where embryos are loaded onto a polypropylene strip and capped before plunging into liquid nitrogen. Significantly higher pregnancies were achieved with the Fibreplug™ (61%, 26/43) compared with the

Cryolock® (30%, 13/43). Of the 39 pregnancies at 20 days of transfer 77% (30/39) resulted from the embryos with a diameter of 300-450μm and 23% from embryos of 500-850μm in diameter. However, the negative effect of embryo size was reduced when embryos were frozen using the Fibreplug™ as pregnancy rates of the embryos ≥ 500 μm were 46% (7/15) and 12% (2/16) for the Fibreplug™ and Cryolock® respectively.

Moulavi et al. (2019) successfully vitrified cloned camel embryos using the Cryolock® carrier system with Cryotec® (Cryotec, Japan) and Kitazato® (Kitazato Corporation, Japan) kits, developed for use with human embryos, and achieved pregnancy rates of 40% and 50% respectively. However, vitrification protocols for embryos produced *in vitro* may not be suitable for those produced *in vivo* due to differences in the chilling and osmotic tolerance of cloned embryos which are much smaller in size than embryos produced *in vivo*.

Conclusion

These experiments have made great advances in the vitrification of camel embryos and have led to the development of a commercially available kit, Herrid's Vitrification Kit® (Minitube, Germany) for camels, that has achieved promising pregnancy rates of 40-45% (Skidmore unpublished data). It is anticipated that this kit will further enhance the commercial application of cryopreservation of camel embryos and help disseminate valuable genetics worldwide.

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Expansion and pregnancy rates of grade 3 folded dromedary camel embryos, following overnight incubation at 37°C

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Introduction

Embryo transfer in the dromedary camel (DC) was successfully developed in the early nineties (Skidmore et al., 1992; McKinnon et al., 1992) and, within the Middle-East, is currently a large scale profitable sector. However, a considerable degree of variability amongst individual donors in response to super-ovulation protocols, along with different *in vivo* fertilization rates, often results in a wide variance in the number of embryos that may be recovered after the flushing procedure (sometimes up to 30); concurrently the number of available recipients may be lowered due to a non response to the ovulation inducing agent or after exclusion for other reasons (i.e. small/poor quality corpus luteum, uterine abnormalities). In such circumstances, when collected embryos are in "excess" with regards to good quality recipients, decisions have to be made in order to maximize pregnancy outcomes whilst optimizing the overall efficiency of the program. In these cases the best embryos (grades 1 & 2) as determined by superior morphological characteristics (spherical, light and smooth appearance with few irregularities in contour), are likely to be preferentially transferred. Conversely, embryos that are designated as grade 3 to 5, due to poor morphological characteristics (folding, darkness, collapsing and developmental retardation), are excluded from transfer (Skidmore, 2005).

It was noticed that folded/collapsed embryos, left for few hours in a holding medium, could undergo morphological changes: including re-expansion and the development of a spherical appearance (Mulligan and Osman, personal observations). The present study was devised in order to establish if these discarded embryos could be recovered and used in an embryo transfer program, following a simple overnight incubation method.

Materials and Methods

Embryo collection and evaluation

Following a superovulation protocol the donors were flushed non-surgically in a standing position at Day 8 or 9 post mating as described by Tibary and Anouassi (1997). Grade 3 embryos that were collapsed and/or extensively folded (Figure 1) were set aside and placed overnight in a non-CO_2, 37°C dry incubator in Vigro holding medium (Vigro Holding Plus, Vetoquinol, Canada). After 22 hours (hrs) of incubation, embryo morphology was again evaluated. Embryos were defined as re-expanded or degenerated and re-expanded embryos were graded spherical or abnormal according to their morphology. Only those embryos that displayed blastocoel re-expansion in conjunction with an excellent spherical morphology (Figure 2) were used. Freshly collected, folded grade 3 embryos, were also transferred, soon after recovery and used as control group.

Treatment of recipients, embryo transfer and pregnancy diagnosis

The recipients were examined by transrectal ultrasonography 24 hrs post-mating of the donors, and those having a follicle 12 to 16 mm in diameter received 20 µg buserelin acetate. After eight days recipients were selected if a 15 mm corpus luteum was detected by ultrasonography along with the absence of uterine abnormalities. Re-expanded or control embryos were transferred into the uterine horn ipsilateral to the corpus luteum of selected recipients, after being loaded into 0.25mm French straws and then into transfer catheters. The tail cocking method was used for assessing pregnancies 7 days post-transfer whereas, at day 45, pregnancies were confirmed by transrectal ultrasonography. The chi-square test was used to compare differences between the 2020-2021 and 2021-2022 breeding season (BS), regarding morphological categories and pregnancy rates.

Results

Overall, 107 grade 3 embryos were cultured in the 2020-2021 BS and 29 were transferred. In the 2021-2022 BS, 195 grade 3 embryos were cultured and 34 transferred. Eleven, freshly collected, folded, grade 3 embryos, were also transferred soon after recovery (control group).

Results for the re-expansion rate and spherical and abnormal morphology, are reported in Table 1: statistical differences, between the two BS were found regarding the percentage

of re-expanded embryos (P<0.001) and the percentages of spherical (P<0.001), abnormal (P<0.001) and degenerated embryos (P<0.001).

Across the two seasons, transferred sperical embryos (n=63) resulted in pregnancy rates of 47.6% and 20.63% at 2 weeks and 45 days respectively (Table 2). In comparison, control embryos (n=11) resulted in pregnacy rates of 63.6% and 9.1% at 2 weeks and 45 days respectively, though the resulting difference was statistically non-significant (N.S.).

Table 1: Morphology of dromedary camel embryos following 22 hours incubation in a non-CO_2 incubator at 37°C during the 2020-2021 and 2021-2022 breeding seasons.

Season	Cultured embryos N	Expanded embryos N (%)	Expanded spherical N (%)	Expanded abnormal N (%)	Degenerated N (%)
2020-2021	107	87 (81.3)	55 (51.4)	32 (29.9)	20 (18.7)
2021-2022	195	41 (21)	34 (17.4)	7 (3.6)	154 (78.9)
		P<0.001	P<0.001	P<0.001	P<0.001

Table 2: Pregnancy rates after transfer of spherical re-expanded embryos following 22 hours incubation in a non-CO_2 incubator at 37°C, during the 2020-2021 and 2021-2022 breeding seasons.

Season	Transfers N	Pregnant 2 weeks N (%)	Pregnant 45 days N (%)
2020-2021	29	17 (58.6)	6 (20.7)
2021-2022	34	13 (38.2)	7 (20.6)
Fresh folded	11	7 (63.6)	1 (9.1)
	N.S.	N.S.	N.S.

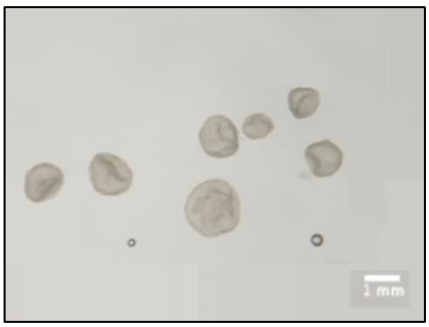

Figure 1: Day 8 folded grade 3 DC embryos

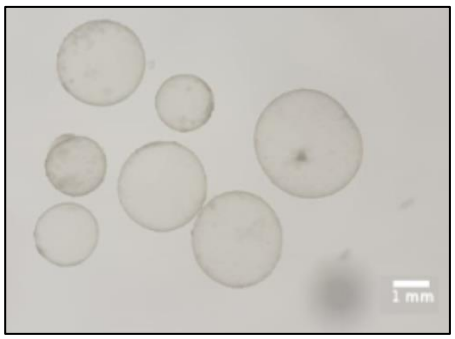

Figure 2: Re-expanded DC embryos following incubation

Discussion

The present study demonstrates that a simple overnight incubation system might be used to recover low grade embryos for transfer and could be used to select re-expanded embryos and to get extra pregnancies from valuable folded/low quality embryos that,

otherwise, would be discarded. The reason of folding/collapsing of embryos is not known but it is of note that some embryos have the ability to improve their morphological characteristics even in holding medium and a non-CO_2 incubator. Transparent, spherical shaped DC embryos display a higher degree of viability with reduced embryonic loss post transfer, in comparison to folded and irregular shaped counterparts (Karen et al., 2022), whereas folded DC embryos undergo a marked reduction in viability when stored at 4°C (Abd Elfattah et al., 2019). Moulavi et al., 2019 stated that the immediate re-expansion of DC embryos is predictive of survival of vitrified cloned DC embryos whereas, in humans, the degree of blastoceol expansion is correlated to pregnancy outcomes during frozen embryo transfer cycles (Zhao et al., 2019). The factors that determined the differences of embryo re-expansion rates across the two breeding seasons remains to be elucidated. The donors' conditions (age, parity, body condition score, superovulation protocols, uterine conditions, number of recovered embryos) could possibly be involved in the embryo competence and re-expansion capability. Whilst no statistical significance was noticed, one pregnacy out of seven was maintained at day 45 (85% of embryonic loss) for fresh folded embryos. In comparison, amongst the re-expanded spherical transferred embryos, 17 out of 30 were lost by day 45 (embryonic loss: 56.6%). More studies are required in order to assess whether the re-expansion observed in the present study, improves the viability of poor quality folded or collapsed DC embryos and more experiments are required for establishing optimal incubation conditions and specific culture media that could improve poor quality *in vivo* derived DC embryo survival rates and viability.

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Superovulation with eCG in dromedary camels

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Introduction

Superovulation (SO) is the key step in multiple ovulation and embryo transfer (MOET) programmes that influences the quantity and quality of harvested embryos. In general, porcine pituitary-derived follicle stimulating hormone (FSH) is used to induce SO in dromedary camels. The FSH is administered twice daily in decreasing or constant doses over a period of 4-7 days [because it has a short half-life of approximately 5 h in cows (Demoustier et al., 1988)], with or without an additional single dose of equine chorionic gonadotropin (eCG) administered at the beginning of the protocol (McKinnon et al., 1994; Anouassi and Tibary, 2013; Skidmore et al., 2002). These protocols require regular handling of the animals by farm-personnel, which increases the possibility of mistakes due to mishandling and errors with administration. To overcome these drawbacks, we have recently developed a simple split-injection protocol using two different FSH preparations (Follitrophin V and Pluset), in which two intramuscular doses (injected 48 h apart) of a slow-release preparation of each FSH (FSH dissolved in 5 % hyaluronan solution), produces a comparable ovarian response and embryo yield to the traditional twice daily FSH protocol (Manjunatha et al., 2019).

Equine chorionic gonadotrophin is a complex glycoprotein with both FSH and LH activity, and has a half-life of 40 h in cows (Schams et al., 1977). A single dose is enough to induce SO in camels (Anouassi and Tibary, 2013). This method of inducing SO is simpler, more economical and also more practical when performing ET programmes in the field, compared with the traditional twice daily FSH injections or a split-injection protocol. The aim of this paper is to provide recent advances in superovulation of dromedary camels with eCG.

Dose of eCG on superovulatory response

In the early days of ET in camels, a single dose of eCG ranging from 1500-6000 IU was used and reported a highly variable ovarian response and embryo yield

(McKinnon et al., 1994; Anouassi and Ali, 1990; Cooper et al., 1992; Skidmore et al., 1992). Later, in a large commercial ET programme, a dose of 1500-3000 IU eCG was used (Anouassi and Tibary, 2013) and then subsequently, a dose of 2500 IU eCG was used (Khalifa et al., 2016). Recently we investigated the effect of different doses (1000, 2000, 3000, 4000, 5000 and 6000 IU) of eCG (Novormon, Syntex S.A., Argentina) on ovarian response and embryo yield (Manjunatha et al., 2020). Based on the number of ovulations, followed by the number of embryos recovered in this study, a single dose of 3000 to 4000 IU eCG was considered as the optimal dose to induce SO in camels. The eCG at low (1000-2000 IU) or high (5000-6000 IU) doses produced a poor superovulatory response.

Timing of eCG treatment after ovulation induction on superovulatory response

Generally, SO protocols in all farm animals are initiated in the absence of large follicles and in the presence of a greater number of actively growing small follicles. In camels, initially the follicular development was synchronized to obtain a mature dominant follicle (DF, 11-17 mm in diameter) which could then be induced to ovulate with GnRH and, thereby, precisely synchronize follicular wave emergence for SO treatments (Manjunatha et al., 2019, 2020). Inducing ovulation with GnRH in the presence of a mature functional DF results in a new wave emergence approximately 3 days later (Manjunatha et al., 2014), but the timing of eCG treatment in relation to follicular wave emergence has not been studied in camels. To investigate the effect of the timing of eCG treatment, after inducing ovulation, on ovarian response and embryo yield, we administered 3000 IU eCG in a single IM injection on either Day 2, Day 3, Day 4 or Day 5 after GnRH (Day 0 = Day of GnRH injection). In this study, the timing of eCG administration after GnRH injection did not affect the number of ovulatory follicles or embryo yield, however, eCG administration on Day 5 affected the ovulatory capacity of the follicles. The results showed that a better SO response was obtained by administering eCG 2-4 days after ovulation induction.

Adverse effects of eCG in supervoulated donors

Superovulation with eCG in cattle has caused protracted ovarian stimulation, ovulation failure, abnormal endocrine profiles, early regression of corpus luteum, and reduced fertilization and embryo quality, possibly due to its long half-life (Mapletoft, 2006). In previous studies that used just eCG for SO in camels, only 52.4-69.3% of them

responded by producing multiple mature follicles (Anouassi and Ali, 1990; Cooper et al., 1992; Anouassi and Tibary, 2013) of which 30-79.2% ovulated (Anouassi and Ali, 1990; Skidmore et al., 1992; Anouassi and Tibary, 2013) and then yielded an average of 2.3-7.1 embryos (McKinnon et al., 1994; Anouassi and Tibary, 2013). In these studies, the eCG (1500- 3000 IU) treatment caused development of two different generations of follicles, premature luteinization of follicles, ovulation failure and development of large anovulatory follicles (Anouassi and Tibary, 2013; Khalifa et al., 2016). In our studies, all camels (n = 80) treated with 3000 IU eCG 3-4 days after GnRH injection, responded with ≥ 5 ovulations and yielded an average of 4.6-5.5 transferable embryos without any adverse effects (Manjunatha et al., 2020). The successful SO achieved in our center could be attributed to the method used for synchronization of follicular wave emergence prior to eCG treatment. However, in our studies high doses of eCG (5000 and 6000 IU) resulted in a high number of un-ovulated follicles, some of which continued growing and became large follicles (≥ 20 mm in size) (Manjunatha et al., 2020). Repeated use of eCG resulted in humoral response to this gonadotropin and has produced anti-eCG antibodies in sheep (Bodin et al., 1997; Roy et al., 1999).

Conclusions

In camels, the optimal dose of eCG to induce superovulation is 3000-4000 IU and it can be administered to induce SO 2-4 days after ovulation induction. Further research in our center will be attempted to examine the repeated use of eCG to induce SO and optimal interval between two eCG treatments in dromedary camels.

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Macroscopic features of dromedary camel placenta at term

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Introduction

The dromedary camel placenta is epitheliochorial, diffuse and microcotyledonary and it has some similarities with aspects of the equines' placenta (Arthur et al., 1985). As for other mammals, a normal anatomical and functional pattern of the placenta is mandatory for the correct development and birth of a viable newborn, and pathologic placentas are associated with several complications of pregnancies (abortion, stillbirth, premature births, fetal hypoxia). Placental evaluation at delivery is, therefore, an essential component of the post-natal evaluation in any species. Findings such as incompleteness, placentitis of infectious or non-infectious origin, oedema or any other abnormality, might provide clues that may not be evident at the clinical examination of the newborn (Carluccio et al., 2008). Notwithstanding, placental evaluation in dromedary camels (post-partum or after abortion) is too often ignored. The post-partum examination of the placenta must be based on knowledge of the normal morphology but, to the best of the authors' knowledge, a complete description of the gross morphology of the placenta is lacking in the dromedary camel species. The present study aims to describe preliminary data regarding the macroscopic features of the placenta of the dromedary camel, after a full-term pregnancy and spontanous parturition, for allowing its first-line on-field examination.

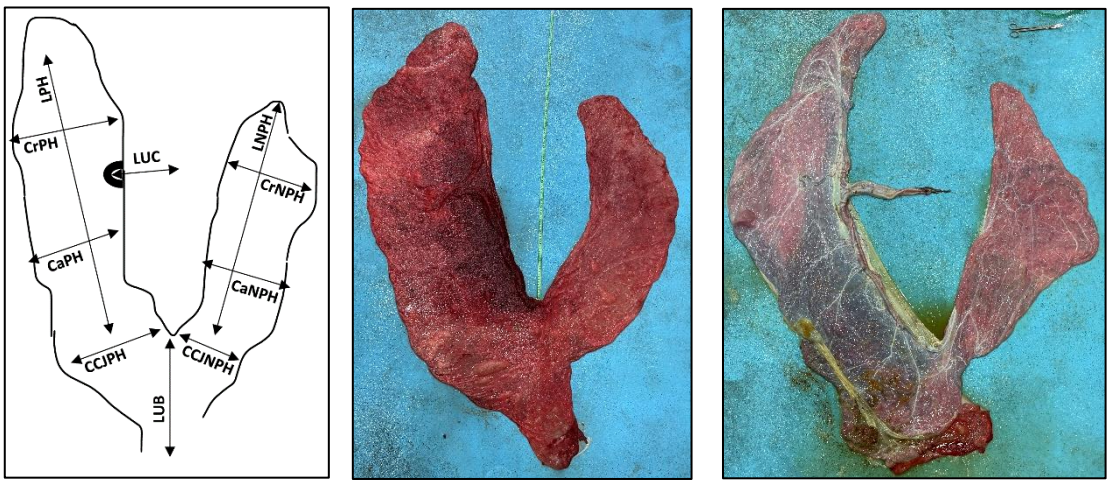
Materials and Methods

The study was performed on she-dromedary camels kept in a free stall barn located in Al-Qassim, Saudi Arabia. Ten intact placentas were collected within 3 h of delivery, from healthy dromedary camel dams (8-15 years old), at term after eutocic spontaneous

parturition. Only placentas from dams giving birth to a single, normal and viable calf, which remained healthy for the first week of life, were selected.

Data about calves' birthweight (BW) and gestational length (GL) were recorded. The placenta was gently cleaned with cold water to remove sand debris, thereafter it was spread on a clean plain surface for the evaluation of the chorionic and allantoic side, respectively. The macroscopic features were evaluated by the following measurements: length of the pregnant (LPH) and non-pregnant horn (LNPH), length of the uterine body (LUB) and of the umbilical cord (LUC); diameter at the corpora-cornual junction of the pregnant horn (CCJPH), and of the non-pregnant horn (CCJNPH), of the cranial middle section of the pregnant horn (CrPH), and of the non-pregnant horn (CrNPH), and of caudal middle section of the pregnant horn (CaPH), and of the non-pregnant horn (CaNPH) (Figure 1). The volume of the pregnant horn (VPH), of the non-pregnant horn (VNPH), of the uterine body (VUB) and of the umbilical cord (VUC) were evaluated by using a graduated bucket, and the total volumes of the placenta (VT) calculated as sum of the parts. The total weight of the placenta (WT) was also measured, by using an electronic scale. The ratio between calves' BW and WT was also calculated as a measure of placental efficiency.

Figure 1: Schematic representation of the dromedary camel placenta measurements (a) (see text for explanation) and evaluation of chorionic (b) and allantoic (c) side.



a **b** **c**

Results

In all the evaluated placentas, pregnancy was established in the left uterine horn. The chorionic side presented a uniform diffused, red, velvety surface whereas the

allantoic side was smooth and white in color. The amniotic sac occupied only the left horn. The umbilical cord contained two arteries and two veins and all specimens presented plaques on the amniotic membrane (allantoamnion) around the umbilical cord. Hippomanes were frequently observed. Descriptive data about the gross placenta measurements, expressed as median (min-max) values, are reported in Tables 1 and 2.

Table 1: Median (min-max) lengths and diameters (cm) of the 10 dromedary placentas.

LPH	CrPH	CaPH	CCJPH	LUB	LUC	LNPH	CrNPH	CaNPH	CCJNPH
154.5 (119-190)	**42** (39-52)	**48** (42-57)	**35** (26-41)	**41.5** (28-51)	**45** (33-53)	**106** (81-136)	**26** (18-33)	**29.5** (21-43)	**22.5** (19-37)

Table 2: Median (min-max) volumes (L) and total weight (Kg) of the 10 dromedary placentas.

VPH	VNPH	VUB	VUC	VT	WT
3.3 (2.5-4.9)	**1.2** (0.9-2.5)	**1.0** (0.6-1.3)	**0.3** (0.2-0.5)	**6.5** (4.7-8.3)	**6.2** (5.0-7.8)

Discussion

In the present study, GL (median, min-max: 374, 357-381 d) was slightly shorter than the mean length reported in dromedaries (384.5±0.17 d; Nagy and Juhasz, 2019) but similar to the median and range reported for donkeys (371.8, 335-395 d) (Carluccio et al., 2021) and to the range reported in horses (301-388 d) (Hecka et al., 2017). Neonatal BW (median, min-max: 44.4, 35.6-47.4 kg) was higher than the 34.5±0.09 kg reported by Nagy and Juhasz (2019) and similar to the mean 40.8±1.8 reported by Atieh et al. (2014) in Saudi Arabia for dams of similar age range, thus probably denoting ecotype differences regarding newborn BW.

The dromedary camel chorionic surface was homogeneous and no avillous areas were detected on the medial side of the horns as reported, in the alpaca, by Meesters et al. (2018). The median weight of the placentas resembles the data reported by Atieh et al. (2014; 6.35±0.43 and 6.51±0.34 kg on average in Majaheem and Wodoh ecotypes, respectively). The ratio between calves' BW and WT was 14%. This ratio is higher than the 11% reported for horses (Whitwell and Jeffcott, 1975; Mariella et al., 2018) and the 12% reported for donkeys (Carluccio et al., 2008), suggesting that the dromedary's placenta could be more efficient than those of equids also considering that GL was more similar to donkeys.

Bravo et al. (2009) reported that the placental efficiency, in alpacas, increased from 8.2% in two years old dams to 9.3% in 6 years old and to 9.5% in 11 years old dams whereas Meesters et al. (2018) reported a 10.3% placental efficiency in alpaca dams aged between 2 and 9 years. In the present study, 10 placentas of camel dams aged between 8 and 15 years where evaluated, therefore a higher number of specimens would be needed for assessing relations between the age of the dam and the efficiency of their placentas. Although preliminary, this is the first study that describes the macroscopic features of the dromedary camel placenta; further investigations, such as histologic examination, are needed for a better description of both normal and pathologic features of the dromedary placenta, but also for better understanding of the real efficiency for the fetal growth and development, as reported for horses and donkeys (Veronesi et al., 2010).

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Pregnancy losses in dromedary camels

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Introduction

Failure of pregnancy at various stages of gestation is a major cause of infertility and economic loss in all livestock species, including the dromedary camel. During the early stages, the embryo or fetus dies and disappears without any obvious external signs. In the past, this condition was called early or late embryonic or early fetal death (Tibary and Anouassi, 1997; Diskin and Morris, 2008). As the transition from the embryonic to the fetal stage of placental development is a gradual process whose boundaries are not precisely defined, recently the term early pregnancy loss (EPL) has been introduced for all pregnancy wastages occurring during the first approx. 100 days of gestation (Pratap et al., 2012; de Mestre et al., 2019). At the same time, pregnancy losses that manifest with the expulsion of a non-viable fetus, such as abortion or perinatal mortality (PM), are considered as mid, or late pregnancy loss (Smith et al., 2003; Mee at al., 2008). Although EPL occurs more frequently, abortions and PM also cause obvious and significant losses. Despite the importance, detailed studies on the epidemiology and etiology of pregnancy wastage are not available for dromedaries and are also scarce in all domestic animal species (Chevalier-Clement, 1989). Moreover, the definition of some pathological terms (i.e., abortion, perinatal mortality) is not standardized, making the comparison of the available studies more difficult. In this review, we summarize our present knowledge both on early and late pregnancy losses that critically influence reproductive efficiency in this species.

Materials and Methods

We conducted studies on various forms of pregnancy loss over 11 breeding seasons from September 2006 through June 2017 at the premises of Emirates Industry for Camel Milk and Products (EICMP), the world's first large-scale camel dairy farm, located

in Dubai, United Arab Emirates (N25º, E55º). During this time, data from 2171 female dromedaries were collected. Early pregnancy losses were monitored during seven breeding seasons from September 2006 through July 2013. In general, camels were between 3 to 24 years of age, had variable parities (1 to 6) and were categorized into six well-distinguishable breeds or ecotypes (Emirati, Emirati-cross, Black, Pakistani, Saudi/Sudanese and Saudi-cross). Further details of farm management including breeding of dromedaries, management of pregnant animals and monitoring parturitions have been described in the published papers (Nagy et al., 2021; Nagy et al.,Theriogenology, 2022 in press).

In our studies, we applied the following definitions. EPL was detected in the first 100 days (d) of gestation. Abortion was diagnosed when a non-viable fetus or fetuses were expelled from the uterus before 330 d of gestation. Whereas PM comprised of three different conditions: **a)** the birth of a premature, non-viable calf after short gestation length ranging from 330 to 350 d; **b)** the birth of a well-developed but dead calf (no heartbeat and respiration) after a normal gestation period; **c)** the birth of a live calf after normal gestation length that died within 48 hours of delivery.

For each pregnancy loss we recorded all relevant general and reproductive information, then univariable and multivariable logistic regressions were conducted to identify risk factors associated with EPL, abortion of singleton fetuses and PM (Nagy et al., 2021; Nagy et al.,Theriogenology, 2022, in press).

Results and Discussion

We monitored EPL in 2970 pregnancies and 507 cases (17.1%) were diagnosed by transrectal examination and ultrasonography. This rate was much lower than what has been reported earlier (30 to 40%; Tibary and Anouassi, 1997; Tinson et al., 2012). The rate of EPL after natural mating and embryo transfer was 16.1% (n = 422 out of 2,616) and 24.0% (n = 85 out of 354), respectively. However, this difference was not significant. Twin pregnancies were detected in 215 cases (7.2% of all gestations), and 57 of those (26.5%) underwent complete EPL. Almost half of the early losses (n = 243; 47.9%) occurred before 30 days of gestation. Another 43.2% (n = 219) of EPL was diagnosed during the next month, and 8.9% (n = 45) occurred after 60 days of gestation. The breeding season (year) and twin pregnancy were the most important exposure variables affecting the rate of EPL ($P < 0.001$). The effect of some male camels was also demonstrated while other factors, such as type of breeding (natural mating vs. embryo

transfer), age category, month of mating, breed/ecotype and reproductive history did not have a significant influence on EPL. We also monitored early pregnancies and EPL in a smaller study at weekly intervals, where the overall rate of EPL was 24.5% (n = 34 of 139). There was no difference in the incidence of EPL between recipient (24.2%, n = 23 of 95) and mated (25%, n = 11 of 44) camels. Weekly rate of EPL ranged from 0.9% to 4.8% with a decreasing tendency, and approx. 41% of the animals (n = 14 of 34) had some ultrasonographic signs of impending EPL one week before the final diagnosis. Day of gestation and future EPL influenced serum P4 levels ($P < 0.001$) with an interaction between the two fixed factors ($P < 0.05$). At the time of the final diagnosis of EPL, mean serum P4 concentration was 2.8 ± 0.44 ng/ml. These results suggests that primary corpus luteum deficiency does not have a role in the etiology of pregnancy loss in this species Although twinning had an unfavorable prognosis with a total pregnancy loss of 36.7%, it was not completely detrimental for the final outcome of gestation as two-thirds of twin pregnancies (n = 136 out of 212) resulted in the birth of a live calf. This study provided no simple solution for reducing EPL in dromedary camels, but highlighted the need for further studies on the relationship between reproductive efficiency and the environment, including climatic and perhaps other astronomical factors (Nagy et al., 2021).

Abortions were detected in 229 cases (5.05%) out of 4533 pregnancies that exceeded 60 days of gestation. This rate is in the lower range compared to published data for this species (2 to 25%; Tibary et al., 2006). Most camels (n = 181, 88.7%) aborted only once and most abortions were singleton (n =199, 89.9%), but twin abortions were also recorded in 30 cases (13.1%). Abortions showed a pronounced seasonal distribution, with a peak in August, but were relatively constant over the years. The age category ($P < 0.01$), breed or ecotype of the female ($P < 0.05$) and bull influenced the occurrence of singleton abortions. Dromedaries with twins tended to abort earlier than those with a singleton fetus (median = 257 d vs. 232.5 d, $P = 0.053$). The variation in gestation length of singleton abortions was mainly related to the month of abortion and to the aborting female.

Perinatal mortality has not been studied earlier in dromedaries. During this study, we observed 174 cases (4.07%) of PM out of 4275 deliveries that exceeded 330 d of gestation. The condition included the premature birth of non-viable calves after short gestation (<350 d, n = 26, 14.9%), the birth of well-developed but dead calves after normal gestation length (n = 120, 69.0%) and neonates that died within 48 hours after delivery (n = 28, 16.1%). The frequency distribution of PM was parallel with that of

parturitions. The most important predisposing factor for PM was difficult calving. Thirty-nine percent (68 out of 174) of these losses were associated with dystocia, and 70.8% (68 out of 96) of dystocia resulted in calf death. In addition, age category ($P < 0.05$) and parity of the female ($P < 0.01$), month of delivery ($P < 0.05$) and breeding season ($P < 0.05$) also affected the incidence of PM. The cause of 60 cases of PM (1.4% of all deliveries) could not be determined and was considered idiopathic.

In conclusion, total pregnancy wastage in dromedaries was rather high as one-quarter of the diagnosed early pregnancies were lost at various stages of gestation. Two-thirds of these losses occurred in the first 100 days, while one-third of total pregnancy losses were detected during mid to late gestation. Approx. 10% of pregnancies that exceeded Day 60 failed, and 90% resulted in the birth of a live calf that survived beyond 48 hours. More than half of these mid to late pregnancy losses were abortions before 330 d of gestation, and approx. 40% were classified as PM. The weekly mean risk of pregnancy loss after 100 d of gestation remained only a fraction of that observed during the first 2 to 3 months. Although, the detailed etiology of abortions and perinatal mortality was beyond the scope of this study, all carcasses were examined and sent for post-mortem examination. Therefore, we may conclude that the diagnosis of these conditions is time-consuming, costly and the cause of death frequently cannot be determined (Nagy et al., submitted).

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Evaluation of relaxin concentrations for pregnancy testing in Alpacas using a stallside test

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Introduction

The number of South American Camelids (mostly alpacas and llamas) in Europe has been increasing steadily over the last 20 years. The UK has the highest population of alpacas in Europe, with approximately 34,000 registered alpacas (BAS Registry, 2020). Alpacas are used primarily for fleece production, although interest in tourism-related activities for both alpacas and llamas has become increasingly popular.

The gestation length of alpacas is approximately 343 days, but has been shown to be highly variable with gestation lengths of up to 375 days being reported. Spring pregnancies tend to be longer while gestation lengths shorten towards autumn (Davis et al., 1997).

There are various methods available for diagnosing pregnancy in camelids including hormonal analysis, rectal palpation, ultrasonography and ballottement. Progesterone concentrations of at least 6 nmol/l indicate the presence of a corpus luteum and is consistent with pregnancy. However, factors other than pregnancy can result in prolonged luteal function, therefore ultrasonographic confirmation of pregnancy should still be performed.

Oestrone sulphate concentrations are a useful indicator of early pregnancy but interpretation relies on accurate breeding records. Concentrations peak twice during pregnancy in llamas and alpacas. The first peak occurs 21-27 days after mating and this peak in oestrone sulphate is thought to be produced by the foetomaternal unit and therefore is only present during pregnancy (Bravo et al., 1996). Plasma concentrations were 44.5 ± 5.4 ng/ml in llamas and 48.1 ± 3.8 ng/ml in alpacas compared with basal concentrations of around 1 ng/ml. A second peak in concentrations occurs during the last 60 days of pregnancy, peaking around 42 ng/ml during the last month before parturition. Oestrone sulphate can also be measured in the urine and concentrations were found to be increased during the last month of pregnancy, but their use in early pregnancy has not been reliably determined (Volkery et al., 2012).

Pregnancy in camelids is confirmed by the use of ultrasonography. Transabdominal ultrasonography can determine pregnancy after 45-60 days, while earlier diagnosis is possible using rectal ultrasound. It is important to confirm maintenance of pregnancy around 3 months prior to expected parturition in order to properly prepare the female for parturition, and subsequent lactation, by feeding her appropriately. Many general practice vets struggle with late gestation pregnancy diagnosis by ultrasound. Readily available hormonal tests to date have included progesterone analysis but since increased progesterone concentration indicates only the presence of luteal tissue, it is not diagnostic of pregnancy.

Relaxin is thought to be produced by an intact foetoplacental unit and analysis after 85 days of pregnancy has been suggested by Bravo et al. (1996). After the first peak at 3.5 months of gestation (>20 ng/ml), concentrations decrease and then increase again from 7.5 months until parturition. Therefore, measurement of relaxin concentrations during certain periods of pregnancy could be useful for diagnosing pregnancy where ultrasound evaluation is unavailable or inconclusive. Unfortunately, laboratory assessment of relaxin concentrations is not readily available as relaxin is an extremely unstable molecule. A point-of-care test has recently been developed for use in dogs and cats (FASTest Relaxin) that would greatly improve diagnostic capability in these cases. However, this test has not been validated for use in alpacas. This study investigates the potential use of this test in alpacas and attempts to validate its use.

Method

Twelve pregnant and six non-pregnant female alpacas, aged between 3 and 9 years, were included in this study which was conducted at a farm in the United Kingdom. Eleven of the females were 89-90 days pregnant and one was 61 days pregnant. All animals were healthy at the time of blood sampling.

FASTest Relaxin is a semi-quantitative point-of-care test for relaxin that is produced and sold by Megacor (Hörbranz, Austria). It is a lateral flow test based on the immunochromatographic sandwich principle. The detection limit of the test is stated by the manufacturer to be 0.5 to 1ng/ml. A study conducted by Schöne et al. (2004), however, defined the cut off at 0.38ng/ml compared to RIA using biotinylated porcine relaxin.

Pregnancy was confirmed by transabdominal ultrasound on the same day as the blood samples were taken. Blood was collected by venipuncture into heparinised tubes and the samples were stored at room temperature until the test was performed.

Within two hours of sampling the blood was centrifuged and the plasma was separated. The FASTest Relaxin test was carried out according to the manufacturer´s instructions, the test kits having been stored at room temperature. Plasma (80-100 µl) and two drops of buffer (80-100 µl) were put into the test well. Reading of test results was performed after 5, 10, 15, 20, 25, 30 and 60 minutes at room temperature. The plasma from one pregnant alpaca was diluted 1:2, 1:4, 1:8

and 1:16 using PBS and heparin-NaCl. The dilutions were then used according to the instructions. The tests were read after 5, 10, 15, 20, 25, 30 and 60 min.

An aliquot of plasma from each animal was also frozen and transported on dry ice to the University of Leipzig three months later for quantitative analysis of relaxin concentrations determined by radioimmunoassay. Samples from a pregnant and a non-pregnant dog were used as positive and negative samples controls on the assay.

Results

The results of the FASTest Relaxin test were all negative, regardless of pregnancy status. By contrast, the stored plasma samples analysed by radioimmunoassay showed relaxin concentrations consistent with those of the canine control sera and showed a clear difference between concentrations in pregnant (mean 4.87ng/ml) versus non-pregnant (0.47ng/ml) alpacas.

Discussion

This study showed that although relaxin concentrations were measurable by standard radioimmunoassay, the point-of-care FASTest Relaxin test was unable to detect alpaca relaxin. Our quantitative results are consistent with those found in two other studies. Volkery et al., (2012) found that relaxin concentrations in alpacas, measured by enzyme immunoassay, increased significantly after two months of pregnancy with mean concentrations of 2.65 and 11.69 ng/ml between 1-60 days and more than 60 days of pregnancy respectively. Bravo et al. (1996), also using radioimmunoassay, found basal concentrations of relaxin during the first two months of gestation, peaking at 3 months and then increasing again after 7.5 months.

The results from the quantitative assay showed a clear difference between pregnant and non-pregnant animals. The reason for the negative results from the test kit is unknown. The most likely explanation is incompatibility of alpaca relaxin “antigens” with the detection and catcher antibodies in the test itself. This is likely to be due to a difference in the relaxin structure of the alpaca since the test is optimized for dogs and cats. Another possible explanation could include differences in the molecular size, charge and physical properties of the alpaca relaxin molecule affecting the flow across the test strip and thereby influencing the analytical result.

The amino acid sequence of relaxin peptides exhibits wide variability across species and, interestingly, closely related species can have high variability while distantly related species may show greater similarity. Further studies could investigate the amino acid sequence and the structure of the relaxin molecule in alpacas in order to examine how binding sites differ, and determine if this would influence the attachment of the antibodies in the lateral flow test.

In conclusion, this study shows that the point-of-care test evaluated is not a useful method for pregnancy diagnosis in alpacas. Further studies are needed to evaluate different antibodies in the lateral flow technology that will be capable of attaching to the alpaca relaxin molecule.

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Use of reproductive ultrasound for fertility investigations of male camels

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Introduction

Fertility of the male is a major contributor to overall reproductive performance and predicting the fertility of males is an area of research that has been active for some time in several species.

Clinical examination, observation of mating performance and behaviour and semen evaluation have been traditionally used to assess males for breeding, with semen evaluation providing the most informative results. However, in some cases the difficulties in training the males to use the artificial vagina to collect semen makes it impractical. Ultrasonography of the testes is another recent development to predict fertility in males (Chapwanya et al., 2008). Ultrasonography is a widely used and well tolerated imaging modality for evaluation of pathologic conditions of the ovaries and testes. Recent technical advances in processing the results have enabled more detailed analyses of the structure and function of testicular tissue which can then be related to male fertility. Some researchers have reported research applications of ultrasonography in female dromedary camels (Skidmore and Adams, 2000; Tibary and Anouassi, 2000), while male assessment has received much less attention.

Recently, several algorithms have been developed to try to quantify the echotexture of ultrasound images of bull testicles according to the distribution of black, white and grey pixels and also according to the size and density of hypoechogenic areas. ECOTEX is a computational analysis of the return intensity of ultrasonographic waves designed to be used with EXAGO scanners. This procedure can help to identify and objectively measure variations that cannot be seen with the naked eye. The quantitative computer algorithms that have been developed for this analysis therefore serve to eliminate the subjectivity inherent in visual analysis. The images are analysed by means

of new algorithms using ECOTEX software. Three parameters are analysed at normal resolution: EC1 (black pixels), EC2 (white pixels) and EC3 (mean grey level) and three parameters, related to hypoechogenic areas (HA) in the ultrasonogram are analysed at high resolution: density of HA/cm^3, % Area of HA and mean diameter of HA.

In our lab we performed two studies involving the use of ECOTEX: **Study 1)** to investigate the relationship between semen quality and *in vivo* fertility capacity of camel bulls and the testicular echotexture parameters provided by ECOTEX, **Study 2)** to investigate the morphological changes in the testicular parenchyma from November to July.

Methodology: ECOTEX

In both studies, the same methodology was performed. Ultrasonograms were carried out using an EXAGO scanner (ECM, France) connected to a 7.5 MHz linear probe, and three transversal ultrasound scans were performed per testicle. In Study 1 (n=15) 10 males used for semen collection and 5 males for natural mating were examined and in Study 2 (n=6) monthly ultrasonograms were carried out in six camels from November to July. The images were analysed by ECOTEX software. Six parameters were analysed EC1, EC2, EC3, density of tubules/cm^3, % lumen and diameter of lumen.

Study 1: Relationship between semen quality and *in vivo* fertility capacity of camel bulls and the testicular echotexture parameters

A semen sample was collected per animal. Sperm motility was analyzed by CASA and a sample was stained with eosin-nigrosin to assess vitality and morphology. A total of 200 spermatozoa were counted per sample and the percentage and type of sperm abnormalities of each sample were determined (head, tail, mid-piece, droplets). The Spearman Correlation Test showed that there was a moderate negative correlation ($p=-0.564$) between density of hypo-echogenic areas and percentage of morphological abnormalities ($p<0.05$) (Table 1).

We used the cut-off point of 20% of total morphology abnormalities to divide the fertile and subfertile camels and this resulted in a population of nine fertile and one sub-fertile male. There were significant differences between the testicles in terms of density of hypo-echogenic areas ($p<0.05$) with fertile males having higher density than sub-fertile.

Table 1: Correlations between sperm parameters and EXAGO parameters.

	EC1	EC2	EC3	HA Area	HA Dia	HA Den
Total Motility(%)	,663	,603	,200	,960	,934	,385
Progressive Motility (%)	,354	,556	,385	,676	,580	,511
Vitality(%)	,500	,474	,082	,612	,893	,108
AI	,395	,475	,475	,776	,815	,290
MA	,323	,214	,111	,285	,367	,041

Moreover, one out of the five males used for natural mating presented a lower fertility rate (33%) when compared with the other males (59-61%). This male also presented the lowest density of hypo-echogenic areas and the highest area and diameter values of hypoechogenic area. This indicates that the density of hypo-echogenic areas may be related to sperm quality in the testicle and fertility. Our results indicated that the density of hypoechogenic areas can be a good indicator of abnormal sperm production and infertility. ECOTEX software has been used in evaluating the testis of several species. For example, evaluation of the hypoechogenic areas in the ultrasonograms of boar (Muñoz et al., 2018) and ram (Gil et al., 2018) testicles have also been used to predict the fertility or subfertility of their semen samples. However, more parameters diverged between fertile and sub-fertile stallions (Lafuente et al., 2018), indicating that testicles producing sub-fertile samples differed significantly with lower values of EC2, EC3, Area and Density.

Study 2: Morphological changes in the testicular parenchyma from November to July

Ejaculates were obtained weekly from each male between November and April. Sperm parameters including, volume, viscosity, concentration, total sperm production, total and progressive motility, vitality, acrosome integrity, morphological abnormalities and ProAKAP4 concentration were assessed. The results indicated that during the reproductive season (November) testicles have a well-developed HA (Table 2). Then, in January there are a significant decrease in HA development, an increase in whiter pixels (Test 2) and an increase in mean grey level (Test 3). In the non-breeding season (March-May), the camel testicles reduced in size and they become very hypoechogenic, with an increase in black pixels. In this context, HA parameters were not reliable and are not therefore shown it in the table.

Table 2: Values of EXAGO parameters per month.

	EC1	EC2	EC3	HA Area	HA Dia	HA Den
November	22,6±70a	10,7±20,9a	76,3±18,7 a	11,6±3,5 ab	108,2±22 a	165,9±8,4ab
January	14,4±37,6a	61,4±108,8b	87,5±15,4 b	9,5±2,3 a	96,0±12,6 a	150,9±10,8a
Mar	864,8±760,9b	4,3±24,1[a]	43,1±18 c			
May	367,2±540c	0,0±0a	51,5±17,8 c			
July	51,1±74,5a	3,3±13,8 a	65,0±19,1 d	14,1±5,9 b	122,5±37,2a	164,3±20,3b

In July testicles recover their size and also the structure of HA and show a development similar to that of November. In the present study, the testicular parenchyma appeared as more echogenic (higher Ec2, Ec3) during the breeding season in comparison to the non-breeding season, as described earlier by Pasha et al. (2011) . The hypoechogenicity found in the ultrasonograms of March and May (very high Ec1), could be related to GnRH depletion which has been described by Pasha et al. (2011) in camels and Ülker et al. (2005) for prepubertal rams. The lack of GnRH causes seminiferous tubule degeneration with loss of cellular density. This phenomenon: a partial tubule degeneration without total cessation of spermatogenesis, has been described for the camel by several authors (Pasha et al., 2011; Zeidan et al., 2013). Another possibility is that this hypoechogenicity is related to the expansion development of the lymph vessels (up to about 10% of intertubular tissue in spring), as described by Zayed et al. (1995). In this study, no differences in sperm abnormalities were found during the period of study, which is in agreement with Al-Bulushi et al. (2019) However, the results showed a tendency for better semen quality in January and poorer semen quality from March to May, which is the period when the bull camels suffer some degree of testicular degeneration. The changes in ultrasound HA preceded the changes in seminal quality by about one month: there was a maximum of HA in November and then a decline in January. In the period from March to May, it was impossible to measure the HA, because of the generalized hypoechogenicity.

Conclusion

This study demonstrated that testicular ultrasonography can be useful for investigating testicular function in dromedary camels. The density of the hypo-echogenic areas seems to be related to the quality of spermatogenesis in the testicle. Further studies involving known fertile and infertile males are necessary in order to conclude that there

is an association between echotexture parameters and semen quality. The seasonality of the testicular parenchyma was analysed by ECOTEX indicating higher activity from December to February and lower activity from March when there is testicular degeneration. To our knowledge this is the first study to describe the prediction model for male fertility using ultrasound in dromedary camels. The incorporation of scrotal ultrasound could be of great interest for the diagnosis and prognosis of the reproductive capacity of dromedary camels.

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Semen collection from dromedary camel bulls, methodology and a new patented approach

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Introduction

Artificial insemination in dromedary camels is considered to have a low overall efficiency and lacks the widespread application seen in other domestic animals. This is due to challenges presented by the semen collection process and the low semen volume and concentration that is observed in this species. Prolonged mating times, mating in sternal recumbency, challenges in bull handling along with complicated mating behaviours are the biggest limiting factors when it comes to reliably collecting a clean semen sample in dromedary camels (Bravo et al., 2000; Deen et al., 2003). Conventionally, two main techniques are utilised for semen collection in camels: (i) with a conventional or modified bovine artificial vagina "AV", and (ii) the electro-ejaculation technique (Musa et al., 1993; Bravo et al., 2000; Skidmore et al., 2013). Both techniques suffer from significant disadvantages when applied to camel bulls and demonstrate a high risk of semen contamination (Tingari et al.,1986; Skidmore et al., 2013). In the current study, we present a newly developed, patented method denoted the "Camel Semen Collection Kit-CSCK", designed to solve the problem of semen collection in dromedary camels. Herein, a comparison between a typically used AV device and the KSKC is presented, with the results of semen parameters collected by either device detailed in this report.

Materials and Methods

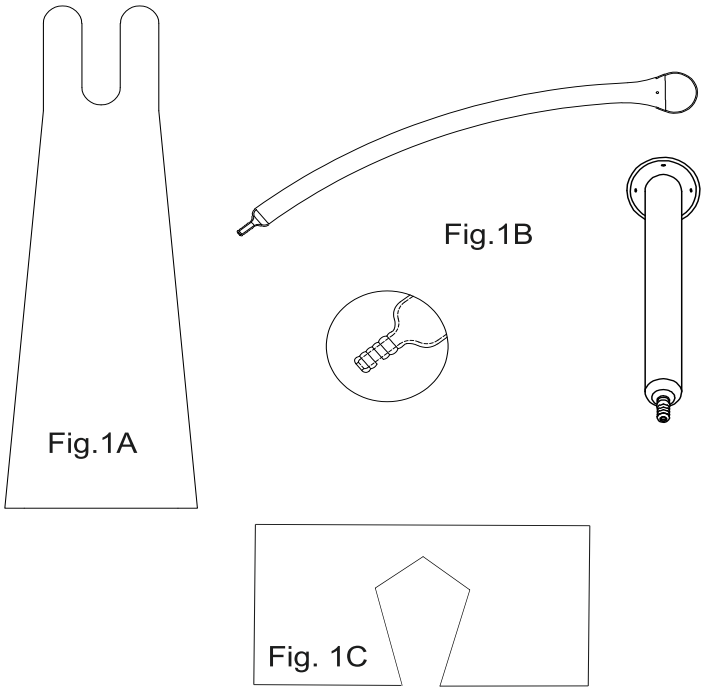
Animals, and semen collection

Eight camel bulls and 25 parous females were used in the current study. A total number of 256 semen collection trials from 8 dromedary camel bulls were conducted from the period of November 2021 to February 2022. Semen was collected twice weekly (on Mondays and Thursdays), alternating between the two methods on either day.

Camel semen collection kit 'CSCK'

The new CSCK (patent reference number: MOE-DIP-92-3341768-20220303) is composed of three main parts: ***(A) Semen collection sac*****:** made from supersensitive, flexible, low-density polyethylene- (LDPE) material with a thickness of 25 µm and a total length of 45 cm. This sac is designed to be introduced deep inside the vagina of the she-camel. Because ejaculation in camels occurs intrauterine and the camel uterus is of a bicornuate type, the collection sac was designed to have a main part which ends with two fingers (Figure 1A); (***B) Metal stainless steel applicator***: at the terminal end of the applicator there is an oval head 4 cm in diameter which gradually tapers toward its rear and contains 4 pores, each 2 mm in diameter. Posterior to the head is a slightly curved cylindrical body which ends with a small pipe, 3 mm in diameter (Figure 1B).; ***(C) Fixation sticker*****:** comprised of a Cushion Sheet Sticker pad which is cut in a rectangular form (18 X 9 cm) with a central ovoid cut (Figure 1C).

Figure 1A: Semen collection sac; **Figure 1B:** Metal applicator; **Figure 1C:** Fixation sticker



To prepare the she camels, trans-rectal ultrasonography was performed in the standing position and only sexually receptive females with a dominant follicle (1.1-1.6 mm in diameter) were used in the collection trials. The perineal region was cleaned, swabbed and disinfected with 70% alcohol. The metal applicator was first sheathed inside the semen collection sac and then inserted into the vagina of a receptive female. A manual

air pump was then connected to the distal end of the applicator and air pumped through the pores in the head of the applicator in order to fixate the collection sac to the vaginal wall of the female. The applicator was then drawn out of the vagina and the fixation sticker was used to secure the outer portion of the collection sac to the female's perineal area. Then the she-camel was taken as normal to the male pen for natural mating. After mating, the semen collection sac was withdrawn from the vagina, cleaned of vaginal secretions from its exterior and then squeezed to collect the deposited semen within.

Modified bovine artificial vagina (AV)

A bovine AV modified for camels with an internal, foam imitation cervix (8 cm in length), designed to stimulate ejaculation was used (Bravo et al., 2000). Preparation of the AV and its use in semen collection was conducted as previously described (Skidmore et al., 2013). Care was taken to ensure that the penis stayed inside the AV during semen collection to decrease the chance of semen contamination occurring.

Success rate and semen evaluation

For either method, both the success rate and duration of semen collection in seconds was recorded. Once semen was collected, the volume, colour and any visible contamination was determined. Gross activity was described as the oscillatory activity of spermatozoa in an undiluted semen sample on a pre-warmed glass slide and scored from 0–3 according to Al-Bulushi et al., (2019). Finally, total motility after liquefaction was measured according to Mansour et al. (2022).

Statistical analysis

A Chi-squared analysis was used to compare differences between the two methods in terms of duration of semen collection, the success rate, rate of visible contamination and semen parameters. The results were presented as mean ± SD. The non-parametric data of semen gross activity was compared using the Kruskal Wallis test and the data are presented as medians. Differences were considered significant at a probability level of $P < 0.05$.

Results

With both methods, collected semen was highly viscous, whitish or greyish-white in colour depending sperm cell concentration. In comparison with the AV, a longer duration of semen collection, higher success rate, greater undiluted gross semen activity

and decreased rates of visible contamination were ($P < 0.05$) recorded with the new CSCK (Table 1). However, sperm concentration and motility following liquification was similar between both semen collection methods (Table 1).

Table 1: Tested semen parameters of dromedary camel collected by AV and CSCK.

Semen parameter	**Semen collection method**	
	Artificial vagina (AV)	**CSCK**
Duration of semen collection/sec	312 ± 192 [a]	585 ± 336 [b]
Successful rate	59/128 (46.1%) [a]	112/128 (87.5 %) [b]
Gross activity	2.0 [a]	3.0 [b]
Visible contamination	23/59 (39.0 %) [a]	11/112 (9.8 %) [b]
Semen volume	4.1 ± 2.7 ml [a]	7.3 ± 3.6 ml [b]
Sperm cell concentration	415.6 ± 112.4 X 10^6 [a]	437.6 ± 62.4 X 10^6 [a]
Sperm motility after liquefaction	56.6 ± 6.1 % [a]	68.2 ± 11.4 % [a]

Values within a column with different superscripts are significantly different (*P < 0.05*)

Discussion

The current study presents a simple and practical method that can be used to collect semen from camel bulls via a new, patented CSCK device which removes the need for manual interference by a handler during the collection process. As an additional highlight, training of the bulls was not required prior to use of the CSCK which likely resulted in the greater success rate of semen collections performed with this system. Previously, typical collections of camel semen have relied upon the bovine artificial vagina (AV) or the bovine electro-ejaculator. To date, the use of a modified bovine AV, which must be held by a handler throughout the collection process, or fixed inside a dummy, is the most routinely used method (Skidmore et al., 2013; Ziapour et al., 2014). Using the electroejaculation technique is highly stressful and complicated by potential negative effects on the bull's health (Tharwat et al., 2014). In the current study, a longer durations of semen collection and greater semen volumes were recorded when the CSCK was used. This indicates that the bulls used in these trials fully accepted the materials used in the CSKC and that the course of mating was not interrupted throughout its use, as can occur with the AV due to its gradual cooling (Ziapour et al., 2014). During natural mating, camel bulls make several thrusts, interspersed by rest periods, and so ejaculation occurs in fractions with the whole process taking between 5 and 20 minutes (Skidmore et al.,

2013). Moreover, amongst the semen samples collected by the AV, a higher rate of visible contamination and a lower semen gross activity was observed relative to the CSCK. The use of the AV is usually accompanied by repeated withdrawals of the penis from the AV throughout semen collection, which acts as the main source of semen contamination (El-Wishy, 1988). Also, the rubber linear of the AV has an adverse effect on semen quality and sperm motility (Musa et al., 1993). In conclusion, the new CSCK represents a practical and easy system that can be used to reliably collect high quality semen from any untrained camel bull. The CSCK may therefore facilitate the widespread application of reproductive technologies such as artificial insemination on a large scale in dromedary camels.

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Problems and solutions associated with camel semen collection and viscosity

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Abstract

Artificial insemination (AI) in camels remains undeveloped due to the difficulties in semen collection, semen viscosity and semen cryopreservation. Semen collection procedures have been facilitated using a camel phantom or possibly an intravaginal condom. Semen viscosity in camelids has been characterized and different mechanical and enzymatic approaches have been used to alleviate this problem; however, there is still no reliable protocol to completely remove viscosity in camel semen. As long as the problem of semen viscosity exists, the problem of semen cryopreservation remains unresolved.

Problems and solutions associated with semen collection in camel

There are several obstacles in the widespread application of AI in camels. The first step in developing AI in this species is being able to collect a clean and viable semen sample. Semen collection in camels is a challenging procedure due to their mating in sternal recumbency, a prolonged time of copulation and possible injuries occurring to the operator. There are four main approaches that can be used, namely: an artificial vagina (Tibary and Anouassi, 1997; Mosaferi et al., 2005), electroejaculation (Tingari et al., 1986), a phantom or dummy (Ziapour et al., 2014) or intravaginal condoms (Tibary and Anouassi, 2018). These methods have certain advantages and disadvantages that can affect semen quality and influence the time taken for collecting semen. The artificial vagina (AV) is the most common approach for semen collection in camels (Skidmore et al., 2020). However, although an AV simulates natural mating, it is tiring and hazardous to the operator mainly due to the prolonged time of semen collection and the position required to collect semen. More importantly, due to several backward and forward movements of the bull during semen collection, the specimen can become contaminated (Ziapour et al., 2014). Electroejaculation is another approach to collect semen from camels (Tingari et al., 1986), however, it is not recommended for routine semen collection

from valuable males. The procedure requires sedation, or even anesthesia, and therefore presents a risk to the life and welfare of the animal. The quality of semen collected by electroejaculation varies in volume and concentration (Tibary and Anouassi, 2018) and can be contaminated with urine. A phantom would be a suitable alternative to a live female. The phantom eliminates the risk towards the operator, thereby facilitating the semen collection procedure and provides more natural conditions to collect good quality, clean semen samples (Ziapour et al., 2014). The chief consideration when using a phantom for semen collection is to be patient when training the bull camels. Once one bull has been trained to use the phantom, it is possible to use this bull to stimulate other bulls to use the phantom for mating, however, it is very important that the bulls are isolated from she-camels throughout the season. Collecting the urine of a she-camel while she is in estrus and spraying it around the back and perineal region of the phantom, and playing a sound record of a she-camel during mating, will also stimulate the bull to think that the phantom is a real camel. The idea of collecting semen by intravaginal condoms is not new (Johnson, 1989), but more recently the use of intravaginal condoms has been revisited for camels (Tibary and Anouassi, 2018). However, the problem of placing the device inside the vagina and firmly fixing it around the vulva, (because of the clockwise and anti-clock wise movement of the penis) are major challenges for this technique to be successful for semen collection in camels.

Problems and solutions associated with camel semen viscosity

Camel semen is viscous in nature and can remain viscous for several hours. The role of this viscosity is not fully understood but it may be required to prevent loss of sperm from the female tract and to protect spermatozoa from a hostile environment. This viscosity creates difficulties in semen collection, processing, cryopreservation and even artificial insemination but regardless, evaluation, processing and cryopreservation of camel semen requires complete liquefaction. The cause of this viscosity and its elimination has been a challenging subject in the world of camelid research. In the early sixties, it was suggested that mucopolysaccharides secreted by the bulbouretral gland may be responsible for camel semen viscosity (Perk,1962). However, recent studies indicated that these chemical compounds, that have been renamed glycosaminoglycans (GAGs), may not be the main cause of the viscosity of camelid semen (Kershaw-Young and Maxwell, 2012; Kershaw-Young et al. 2013). There is now supporting evidence to indicate that proteins within seminal plasma, such as mucin, may be responsible because

the viscosity can be reduced by adding cysteine protease enzymes to the semen such as Papain, present in Papaya (Kershaw-Young et al., 2013, 2017), or Ficin, present in Figs (Keshavarz et al., 2016). Following Ficin treatment and centrifugation, a round pellet formed at the bottom of a conical tube and it was easier to separate the supernatant cleanly from the pellet. However, in untreated and centrifugated samples, an oblique, sticky pellet formed along the wall of the tube which stuck to the supernatant during withdrawal (Keshavarz et al., 2016). The positive and negative effects of enzyme treatment on semen happens fairly quickly. Thus, the time required for the action of the enzyme to remove semen viscosity is similar to the time taken for that enzyme to adversely affect the sperm quality. Therefore, the action of the enzyme has to be neutralized after exposing it to the semen by adding an enzyme inhibitor. This procedure did not seem to be detrimental to spermatozoa function (Malo et al., 2017b; Kershaw-Young et al., 2017). Alternatively, camelid sperm can be extracted from semen without enzymatic treatment using a combination of gentle pipetting and single layer centrifugation without detrimental effect on sperm quality (Malo et al., 2017a; Morrell et al., 2021).

Apart from the enzymatic approach to reduce semen viscosity, mechanical and ultrasonic approaches have also been investigated. Stirring of camel semen with paper clips, on very low speed (150 RPM) for 15 minutes (Mosaferi et al., 2005), gentle pipetting of semen in a diluent (Morton et al., 2008) and passage of semen back and forth through a needle have also been used to reduce semen viscosity (Santiani et al., 2005). More recently, it was proposed that dromedary camel semen exposed to ultrasound waves (40 KHz) for 2 min, interspersed with a 2 min break and repeated 4 times could reduce semen viscosity without having deleterious effect on semen viability (Rateb, 2016). While all of these methods could be simple and fairly effective, they do not completely eliminate semen viscosity. Thus, further research is necessary to find an effective method to reduce semen viscosity that does not have a detrimental effect on the sperm.

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Colloid centrifugation of alpaca semen

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Introduction

Despite continued interest in the potential development of artificial insemination (AI) in South American camelids, particularly alpacas and llamas, progress has been slow. The challenges to overcome in semen processing are mainly related to the viscous ejaculate and low sperm concentration that are characteristic of camelid semen. Together these factors contribute to produce sperm samples that are difficult to handle and almost impossible to aliquot for controlled experiments. These andrological issues are just the start; female camelids also present a unique set of challenges that are hindering the development of AI, but these are beyond the scope of the present review. The purpose of this mini-review is to provide an update on processing of alpaca semen by colloid centrifugation.

Semen collection methods

Various methods of semen collection were reviewed recently (Abraham et al., 2017). Since then, pharmacological methods based on methods used successfully in horses and adapted for alpacas were reported (McAllister et al., 2021). Unfortunately, although secretions were obtained following administration of some of these agents, they proved to be azoospermic.

Semen processing

Promising results have been obtained using centrifugation of extended semen through a colloid, adapting the method using one layer of colloid (Single Layer Centrifugation; SLC) reported for other livestock species (Morrell & Rodriguez-Martinez, 2009). The adapted SLC was used with llama semen after a short treatment with enzymes (Trasorras et al., 2012; Santa Cruz et al., 2016). It was subsequently used without enzyme treatment for semen from dromedary camels (Malo et al., 2017; 2018)

and for llama semen (Bertuzzi et al., 2020). The results with dromedary camel and llama semen are interesting as offspring were produced following AI with the processed semen in both species. Since previous attempts to inseminate frozen/thawed dromedary camel semen were largely unsuccessful, the production of offspring after insemination with SLC-processed thawed sperm samples are particularly noteworthy.

Method in brief

The key to this method is to initiate break-up of the viscous seminal plasma as gently as possible, preferably by prolonged gentle pipetting in an excess of warm (37 °C) semen extender using a plastic Pasteur-type pipette. The semen extender presumably helps to protect the spermatozoa during this process, since better results were produced than previous reports with "needling" – repeatedly drawing the semen though a needle. Once the viscous seminal plasma is broken down, the second important step is to separate it from the spermatozoa as soon as possible, otherwise the viscosity returns and the spermatozoa are trapped again.

The SLC was carried out using a low-density colloid achieved by diluting the colloid used for dromedary camel semen 1:1 (v/v) with semen extender. After carefully pipetting the semen sample on top of the colloid, the tube was centrifuged at 300 g for 20 min, the supernatant was removed and the sperm pellet resuspended in fresh semen extender. After evaluating the sperm quality, these samples were either cooled and stored overnight at 5 °C or were frozen after the addition of cryopreservation medium. The results showed that: **i)** spermatozoa could be liberated from the seminal plasma by incubation and gentle pipetting. **ii)** sperm motility and membrane integrity were greater in the SLC samples than in controls, both immediately after processing and after overnight storage at 5 °C. Sperm morphology and membrane functionality, as measured by the hypoosmotic swelling test, were not different between treatments.

iii) sperm motility and plasma membrane integrity were substantially reduced after cryopreservation but mean values were higher in the SLC samples than in controls (Morrell et al., 2021).

Thus, although the method for extracting spermatozoa from a viscous ejaculate was successful and the SLC sperm samples were of better quality than controls both initially and after cooled storage, more work is needed to optimize the cryopreservation protocol for these samples.

These results are promising because it was possible to extract the spermatozoa from the viscous seminal plasma without damaging them. It is still not clear whether the use of enzymes to break down the seminal plasma (Kershaw et al., 2017) is detrimental to sperm survival, and therefore the development of a technique for sperm extraction that does not include enzyme treatment is interesting. The ability to store the sperm samples for 24h facilitates their use in AI, since the processed samples could be transported to other locations where there might not be access to a semen processing laboratory. However, the poor quality in thawed samples was disappointing. A functioning method for sperm cryopreservation would be of considerable benefit in the development of AI in this species, but more research is needed to produce a functional protocol for alpaca sperm samples.

Previous attempts to cryopreserve alpaca semen produced similar results, in contrast to freezing of llama semen, which produces sperm samples of acceptable sperm quality for reproductive biotechnologies. Offspring have been produced from AI (dromedaries) or after transfer of embryos derived from in vitro fertilization of llama oocytes with thawed sperm samples (Fumoso et al., 2019), so theoretically it should be possible to cryopreserve alpaca sperm samples successfully and produce offspring after AI. The relative lack of success to date in producing offspring after AI with cryopreserved camelid sperm samples may be due to the high levels of fragmented DNA seen in cryopreserved llama sperm samples (Fumuso et al., 2019) or to extensive ultrastructural damage occurring during cooling and freezing (Zampini et al., 2020). In this case, extender additives directed towards enhancing sperm protection or providing antioxidant activity (Santiani et al., 2013) could be beneficial.

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Seasonal variations on morphometry and abnormalities of alpaca (*Vicugna pacos*) spermatozoa

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Introduction

Ejaculates of South American camelids are characterized by a high degree of variability amongst individual animals. The semen parameters of alpacas may be impacted by environmental and farming conditions, seasonal effects (rainy and dry season) as well as excessive thermal stress. The aim of this study was to evaluate the effect of two seasons (summer and winter) on morphological characteristics of spermatozoa from alpacas housed in pens and at sea level.

Materials and Methods

Semen from four male alpacas was obtained at the Laboratory of Animal Reproduction, Faculty of Veterinary Medicine, Universidad Nacional Mayor de San Marcos (Lima, Peru). The ejaculates were collected by means of an artificial vagina during the summer (February and March) and winter (August and September) seasons respectively. To reduce thread formation, all raw semen samples were processed by a previously described mechanical method (Bérgamo et al., 2015). Sperm cell morphology (abnormalities and morphometry) was assessed on slides stained with eosin-nigrosin (Aisen et al., 2015), under an inverted Nikon Ti-S microscope. Images were processed with Nikon NIS Elements Advanced Research microscopic image processing software and morphology parameters were analyzed statistically by an ANOVA test, with a Fisher–LSD post hoc test, using the software StatSoft, Inc. (2007).

Results

Whilst moderate variations of morphological characteristics were observed amongst the individual males no interaction was shown with the season. Table 1 displays the sperm cell morphology and morphometry results. During the summer, the percentage of morphologically normal sperm cells was 64.13±3.22% (mean ± S.E.), whereas in winter it was 54.77±1.91% ($p<0.05$). Sperm cell morphometry analysis showed the following values: 6.02±0.05 µm vs. 6.27±0.06 µm for sperm head length ($p<0.001$); 3.44±0.038 µm vs. 3.51±0.04 µm for sperm head width ($p<0.05$); 1.78±0.024 and 1.81±0.03 for ellipticity of sperm heads (n.s.); 16.78±0.22 μm^2 vs. 17.72±0.25 μm^2 for area of sperm heads ($p<0.001$); 10.47±0.28 µm vs. 6.87±0.13 µm for length of middle pieces ($p<0.0001$) and 41.86±0.19 µm vs. 43.11±0.24 µm for length of sperm tails ($p<0.0001$) for Summer and Winter periods respectively.

Table 1: Effect of season on alpaca sperm cells morphology and head morphometry.

	SPERM CELL MORPHOLOGY (%)			HEAD SPERM CELL MORPHOMETRY			
Season	**Normal**	**Detached normal head**	**Bent tail**	**Length (µm)**	**Width (µm)**	**Ellipticity**	**Area (μm^2)**
Summer	64.13±3.22	0.46±0.31	0.0±0.0	6.02±0.05	3.44±0.038	1.78±0.024	16.78±0.22
Winter	54.77±1.91	2.57±0.46	1.38±0.29	6.27±0.06	3.51±0.04	1.81±0.03	17.72±0.25
p value	0.05	0.02	0.015	0.001	0.05	n.s.	0.001

n.s.: not significant

Discussion

The results obtained in this study confirm that there are indeed seasonal variations in sperm cell parameters observed in alpacas. These seasonal variations seem to exist even when the animals are housed at sea level and not exposed to the strong environmental changes occurring in the highlands. On the plateau, during the rainy season (November to April) a higher percentage of normal spermatozoa, along with lower percentages of spermatozoa with head and tail abnormalities can be seen in alpaca ejaculates (Huanca et al., 2011). The sperm head morphometry values obtained in this study were similar to those reported by Buendía et al. (2002). However, the normal sperm percentages were lower than those reported by Flores et al. (2002). Giuliano et al., 2008, reported that llama ejaculates collected with an artificial vagina during either winter or summer periods showed similar percentages of normal sperm cells and those displaying tail abnormalities

It may be concluded that during the summer, alpaca semen parameters are characterized by a higher percentage of normal sperm cells, a reduced size of the sperm head and a greater length of the middle piece. These improved morphological parameters could be related to a better fertilizing capacity of alpaca spermatozoa during the summer.

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Use of seminal plasma to induce ovulation in alpacas (*Vicugna pacos*) to improve embryonic survival on day 5 or 7 after mating and pregnancy rates in embryo recipients

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Introduction

Alpacas, like other camelids, are classified as induced ovulators (San Martin et al., 1968) and require an external stimulus, mating or treatment with an exogenous hormone to induce ovulation. A protein present in the seminal plasma (SP) identified as β-nerve growth factor (β-NGF) is responsible for ovulation induction and corpus luteum formation (Adams et al., 2005; Kershaw-Young et al., 2012). The use of purified β-NGF has been reported, but under Peruvian field conditions, seminal plasma from semen collections that are not suitable for artificial insemination can be used for other studies that require ovulation induction. Alpacas exhibit poor reproductive efficiency attributed to a high embryo mortality before 35 days post-mating (Huanca et al., 2012, Tibary, 2001). Therefore, the continued refinement of reproductive biotechnologies, such as embryo transfer, will better assist genetic improvement in alpacas (Sumar, 2013; Vaughan et al., 2013). The aim of the current study was to evaluate the effects of SP on embryo survival on day 5 or 7 post-mating and pregnancy rate in alpaca embryo recipients.

Materials and Methods

The experiment was performed on 6-8 year-old, non-lactating adult female Huacaya alpacas (n=122) at La Raya Research Station in the Department of Puno-Perú from February to April. Alpacas were selected when a follicle ≥7 mm was detected by transrectal ultrasonography using a 5.0 MHz transducer and an ALOKA SSD 500 and then assigned randomly to one of two experiments.

Experiment 1

Eighty-four alpacas were assigned randomly to one of three groups. Each alpaca was injected intramuscularly (IM) with either 1 mL SP on day 5 (SPD5; n=28) or day 7 (SPD7; n=28) post-mating or 1 mL of PBS (PBS; n=28) on mating day. Animals were evaluated for pregnancy diagnosis by ultrasound on day 25 and 35 post-mating.

Experiment 2

Thirty-eight alpaca embryo recipients were assigned randomly to one of two groups. Ovulation was induced using either 1 mL SP IM (n=20) or 8 μg GnRH IM (n=18). Each female then received an embryo of similar quality on day 7 after ovulation induction. Alpacas were evaluated for pregnancy diagnosis by ultrasound on day 25 post-embryo transfer (day 32 after induction of ovulation).

Data from both experiments were analyzed by chi-squared test.

Results

Experiment 1

There was no significant difference in pregnancy rate among groups on days 25 and 35 post-mating (P>0.05; Table 1).

Table 1: Pregnancy rate at day 25 or 35 post-mating following intramuscular injection of seminal plasma (SP) on day 5 or 7, or saline (PBS) on day of mating.

	Day 25 (%)	Day 35 (%)
SP Day 5	75.0 (21/28)	67.9 (19/28)
SP Day 7	78.6 (22/28)	78.6 (22/28)
PBS Mating day	67.9 (19/28)	60.7 (17/28)
Total	**73.8 (62/84)**	**69.0 (58/84)**

Experiment 2

There was no significant difference in pregnancy rate among groups 32 days after induction of ovulation using SP or GnRH, and 25 days after embryo transfer (P>0.05; Table 2).

Table 2: Pregnancy rate in alpaca embryo transfer recipients 32 days after induction of ovulation using seminal plasma (SP) or GnRH.

Treatment	Day 25 (%)
SP	70.0 (14/20)
GnRH	66.7 (12/18)
Total	**68.4 (26/38)**

Discussion

Seminal plasma does not produce a significant improvement in pregnancy rate following injection on day 5 or 7 after mating compared with the control group. These results suggest that there are other factors involved in early embryonic death in alpacas which require further research.

Seminal plasma, like GnRH, is effective at inducing ovulation in alpacas. The use of seminal plasma to induce ovulation in recipient alpacas produces a similar response to GnRH and can be used in embryo transfer programs.

Acknowledgment

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Unveiling the luteotrophic role of beta-NGF present in the seminal plasma of llamas

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Abstract

The well-established ovulatory effect of NGF mediated by the release of the preovulatory LH surge from the pituitary gland is determinant for the initial stage of luteinization and CL formation and function in llamas. The formation of the CL by intrauterine infusion or intramuscular administration of β-NGF consistently results in higher progesterone output from early stages of CL development than those induced after GnRH administration. Moreover, a positive relationship has been established between the magnitude of the LH peak and the following luteal function when females are treated with either β-NGF purified seminal plasma or whole seminal plasma. In addition, it was observed that both circulatory concentration of NGF and LH increased during the first 3.5 h after administration of β-NGF. Therefore, we cannot rule out a local effect of β-NGF at the ovarian level that could potentiate progesterone production. Indeed, the luteotrophic effect of β-NGF was associated with enhanced tissue vascularization during the preovulatory period and early stages of CL development enhancing steroidogenesis. These vascular changes have been associated with a direct effect of β-NGF on VEFG in llama granulosa cells collected from preovulatory follicles. *In vitro* application of NGF to llama preovulatory granulosa cells leads to an increase of the expression of genes related to progesterone production and angiogenesis. We conclude that the luteotrophic effect of β-NGF in llamas is related with the magnitude of LH secretion and a local ovarian effect in the granulosa cells of the preovulatory follicle enhancing corpus luteum formation and progesterone secretion.

Introduction

Ten years ago, two studies (Ratto et al., 2012, Kershaw et al., 2012) published for the first time that the neurotrophin beta-nerve growth factor (β-NGF), present in the

seminal plasma of llamas and alpacas, was the molecule responsible for inducing ovulation in these species. Since then, several investigations have been conducted to elucidate the potential mechanisms of action of this growth factor at the hypothalamus-hypophysis-gonadal axis (Silva et al., 2011; Carrasco et al., 2018) and at the local level of the ovary (Valderrama et al., 2019, 2020). We know that β-NGF is not only a potent ovulatory molecule but also has a luteotrophic effect, as systemic or intrauterine infusion of the neurotophin affects the diameter of the corpus luteum (CL), and increases the area of CL vascularization followed by a significant increase in progesterone secretion when compared to GnRH-treated females. We believe that the luteotrophic effect is related to: **i)** a unique pattern of NGF-induced LH secretion, and **ii)** a local ovarian effect at the level of the preovulatory follicle, enhancing the expression of steroidogenic enzymes the angiogenic Vascular Endothelial Growth Factor (VEFG).

LH secretion pattern induced by β-NGF: a potential central role of the luteotrophic effect in llamas

The luteogenesis process has been studied in more detail in spontaneously ovulating species; in the bovine model, the main events associated with final follicle differentiation are the acquisition of LH receptors in granulosa cells (Xu et al., 1995) and an acute increase in mRNA expression of steroidogenic enzymes. The most important feature during the periovulatory period is that follicular steroidogenesis shifts from predominantly estrogen/androgen to progesterone production. Several llama studies conducted by our laboratory (Adams et al., 2005; Tanco et al., 2011; Ulloa-Leal et al., 2014) have consistently described a higher progesterone secretion in NGF-treated than that of GnRH-treated females. The preovulatory LH surge induced by NGF is of a greater magnitude than that observed in GnRH-treated females. Indeed, mean values of LH never reach baseline concentrations at 8 hours of sampling period compared to the control group. The magnitude of the NGF-induced LH release has been associated with the development of a CL able to secret more progesterone than those following GnRH treatment (Tanco et al., 2011). Also, a significant increase in the vascularized area in the preovulatory follicles and CL of NGF-treated females using Power Doppler ultrasonography has been described (Ulloa-Leal et al., 2014). This increase in CL vascularization was confirmed by histological analysis (Silva et al., 2017). In a further llama study, Valderrama et al. (2019, 2020) described that systemic administration of NGF up regulates the expression of StAR, cytochrome P450 side-chain cleavage

(P450scc or CYP11A1), 3b-hydroxysteroid dehydrogenase (HSD3B), and the angiogenic factor VEGF in granulosa cells collected from preovulatory follicles in llamas.

Systemic β-NGF: A potential local role of the effect in llamas

Along with the LH effect on the luteogenesis process, we cannot rule out a local effect of β-NGF at the ovarian level that could potentiate progesterone production/secretion. A study conducted in llamas (Berland et al., 2016) documented that ovulations were observed in those females mated with an intact male or after intrauterine infusion of seminal plasma whereas no ovulations were found in those llamas mated with an uresthrostomized male or treated with phosphate-buffered saline. In the same study, plasma NGF concentration increased significantly in only those ovulated females, and it was positively correlated with an increase of plasma LH concentration. The highlights of this last study: **i)** NGF is the chemical signal to induce ovulation in llamas, **ii)** NGF crosses the endometrium and enters the circulatory system rapidly after natural mating or intrauterine infusion of seminal plasma, and **iii)** this allows NGF to influence ovulation and luteogenesis both systemically and at the local level of the ovary. In this sense, expression of both TrkA and $p75^{NTR}$ NGF receptors in granulosa and theca cells have been found in rabbits (Maranesi et al., 2018), cows (Carrasco et al., 2016), rats (Dissen et al., 2000), and humans (Dissen et al., 1996) suggesting that the action of β-NGF may also be exerted directly at the ovary. Consistent with this notion, the supplementation of NGF to a primary culture of llama granulosa cells up regulated VEGF and steroidogenic enzyme-driven progesterone secretion, *and* the addition of NGF to these cells promoted the secretion of progesterone into the culture medium (Valderrama et al., 2019, 2020).

Conclusions

We conclude that β-NGF is the main molecule responsible for induction of ovulation in llamas and alpacas. When it is given by intramuscular or intrauterine infusion, it exerts a potent action in these species. This effect is associated with the LH secretion pattern induced by NGF and by a local action at the ovarian level. Systemic administration or *in vitro* supplementation of NGF in granulosa cell culture results in upregulation of the angiogenic factor VEGF and steroidogenic enzyme-driven progesterone secretion.

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Effect of seminal plasma on the retrieval rate and quality of cumulus-oocyte complexes from ovum pick up in alpacas (*Vicugna pacos*)

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Introduction

Alpacas, like other camelids, are induced ovulators and require an external stimulus to induce ovulation (San Martin et al., 1968). A protein present in the seminal plasma (SP) is responsible for stimulating ovulation (Adams et al., 2005) and has been identified as beta-nerve growth factor (β-NGF; Kershaw-Young et al., 2012). In one study, the interval between the application of SP, GnRH or mating to the occurrence of ovulation was 26 h (Huanca, 2015), a time similar to that reported by Stuart et al. (2015) but using different NGF-β concentration. However, limited information exists on *in vivo* maturation (IVM), retrieval rate and quality of cumulus oocyte complexes (COCs) induced with SP, and subsequent use in *in vitro* fertilization (IVF). The aim of the current study was to evaluate the effects of ovulation inducers SP and buserelin acetate (GnRH) for the synchronization of follicular emergence and ovarian stimulation with equine Chorionic Gonadotropin (eCG) for the recovery of the COCs by ovum pick-up (OPU).

Materials and Methods

The experiment was performed on 5-8 year old, non-lactating adult female Huacaya alpacas (n=60) at the Quimsachata Research Station in the Department of Puno-Perú during January and February. Alpacas were selected when a follicle ≥7 mm was detected by transrectal ultrasonography and assigned randomly to two groups of 2x2 experimental design.

Experiment 1

Twenty-nine alpacas were assigned randomly into two groups in which ovarian synchronization was induced using 1 mL SP IM (n=15) or 8 μg GnRH IM (n=14). They

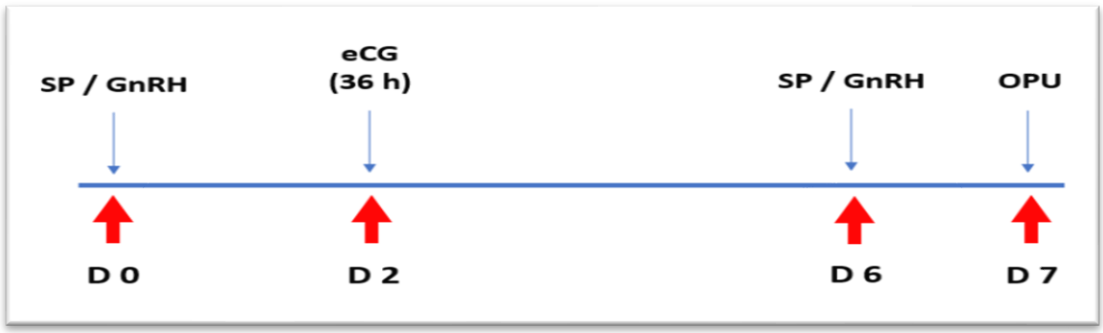
were super-stimulated 36 or 48 hours later using 650 UI eCG IM. Alpacas were examined by ultrasonography at day 7 to count the number of follicles ≥4 mm present (Figure 1).

Figure 1: Treatment design used in Experiment 1.

Experiment 2

Thirty-one alpacas were assigned randomly into two groups in which ovulation was induced using 1 mL SP IM (n=16; G1) or 8 μg GnRH IM (n=15; G2) and then super-stimulated 36 hours later using 650 UI eCG IM. On day 6, alpacas were subdivided into two further groups: G1a: Application GnRH (n=6); G1b: Application SP (n=10); G2a: GnRH (n=8); G2b: SP (n=7). On day 7, OPU was performed to recover COCs, which were then evaluated (Figure 2).

Figure 2: Treatment design used in Experiment 2.



Quality of COCs was based on the number of cumulus cell layers and cytoplasmic homogeneity. They were classified as excellent (A), good (B), regular (C) and degenerate (D) according to Wieczorek (2020).

Ovulation was determined by disappearance of the previous dominant follicle in both experiments. Data were analyzed using ANOVA.

Results

Experiment 1: There was no difference in follicle number between females synchronized and superstimulated at 36 or 48 h, 7 days after eCG treatment (Table 1).

Table 1: Number of follicles ≥4 mm at day 7 by ultrasonography.

	36 h	48 h
Seminal plasma	11.75 ± 1.93 (n=8)	10.00 ± 1.57 (n=7)
GnRH	11.50 ± 2.16 (n=6)	11.25 ± 2.18 (n=8)
Total	**11.64 ± 1.4 (n=14)**	**10.67 ± 0.6 (n=15)**

Experiment 2: COCs were obtained from follicles ≥4 mm in 31 alpacas. The percent of follicles aspirated was 81.64% (369/452), but COC recovery rate was 37.40% (138/369). Table 2 outlines quality of COCs recovered from each group. There was no statistical difference (P>0.05) among groups.

Table 2: Quality of COCs recovered by Ovum Pick-Up.

HORMONS (2X2) N° of Alpacas		**QUALITY OF COCs RECOVERED**				**TOTAL COCs**
Induced	**Maturation**	**A**	**B**	**C**	**D**	
SP **G1 (n=16)**	SP **G1b (n=10)**	10.14% (n=14)	5.80 % (n=8)	4.35% (n=6)	0% (n=0)	**20.29%** (n=28)
	GnRH **G1a (n=6)**	9.42% (n=13)	7.97% (n=11)	0.72% (n=1)	0% (n=0)	**18.11%** (n=25)
GnRH **G2 (n=15)**	SP **G2b (n=7)**	11.59% (n=16)	8.71% (n=12)	2.17% (n=3)	0% (n=0)	**22.47%** (n=31)
	GnRH **G2a (n=8)**	15.94% (n=22)	13.77% (n=19)	9.42% (n=13)	0% (n=0)	**39.13%** (n=54)
TOTAL (n=31)		**47.09%** (n=65)	**36.25%** (n=50)	**16.66%** (n=23)	**0%** (n=0)	**100% (n=138)**

Discussion

Seminal plasma, like GnRH, had an effect on the hypothalamic nuclei to induce pulsatile LH secretion and induce ovulation. The results suggest that the interval from induction of ovulation to super-stimulation in alpacas can be 36 or 48 hours as all groups

resulted in similar retrieval rates and COC quality. We suggest further studies to evaluate the fertilization capacity of the recovered oocytes and their subsequent embryonic development.

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Selenium supplementation in camels using a depot injection of barium selenate

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Introduction

When camels are kept under intensive conditions for racing or milk production, it is important to meet energy, protein, fibre, vitamin and mineral requirements to optimise production. Selenium is one of the essential trace minerals required by camels (Faye and Seboussi, 2009). It is a constituent of glutathione peroxidase, an intracellular enzyme that protects cells from oxidative damage during normal cellular metabolism by catalysing the removal of hydroperoxides. In excess, selenium is toxic as its reduction produces the reactive oxygen ion, superoxide, which can cause lipid peroxidation of membranes, DNA damage and irreversible denaturation of essential cellular proteins, resulting in cell damage and necrosis (Letavayova et al., 2006).

Adequacy of selenium in the diet can be determined by blood testing (McDonald et al., 2002). Supplementation of selenium-deficient camels can be undertaken by fortifying feed or offering mineral blocks, but it is difficult to guarantee an accurate dose in every camel if they are housed and fed in groups.

A depot selenium product has been developed for use in selenium-deficient cattle. Barium selenate solution is delivered by subcutaneous injection annually, based on body weight, and could provide a method of delivering an accurate dose of selenium to deficient camels. This paper describes response to treatment with barium selenate by a group of selenium-deficient camels.

Materials and Methods

Twenty non-pregnant, non-lactating adult female Dromedary camels in body condition score 2.5-3.5 (out of 5), were selected for this study in the United Arab Emirates. They were estimated to weigh 400 kg but no scales were available to more accurately determine this. The camels were housed together in a sand-based yard and

offered daily 1 kg/camel CRC Mix® concentrate (Zabeel Feed Mill), and Rhodes grass hay and water *ad libitum* for the duration of the trial.

Blood was collected from each camel by jugular venepuncture using an 18G, 1.5 inch BD Vacutainer® (Becton, Dickinson & Co., USA) needle into a 10 mL plain BD Vacutainer® tube and submitted to Central Veterinary Research Laboratory for serum selenium assay (CVRL/AC 003-13; APHA-3113B; normal reference range 1.5-2.6 µmol/L) on a monthly basis for 12 months.

Eleven camels had a serum selenium concentration < 1.5 µmol/L at the initial blood test. They were injected subcutaneously 8 days later with 200 mg Selovin LA® (Bayer Australia, 50 mg Se/mL as barium selenate), based on the cattle dose of 0.5 mg/kg. Nine camels had a serum selenium concentration ≥ 1.5 µmol/L at the initial blood test and remained as untreated controls.

Results are presented as mean ± SEM.

This trial was carried out under the direction of the Camel Reproduction Centre Animal Ethics Committee.

Results

Figure 1 shows the response to treatment with barium selenate. All camels that were deficient at the first blood test exhibited serum selenium concentrations > 1.5 µmol/L at the second blood test, and remained in the normal range for the duration of the trial. By the end of the trial, the control group mean was 1.44 (± 0.13), and 6 of 9 camels were selenium deficient.

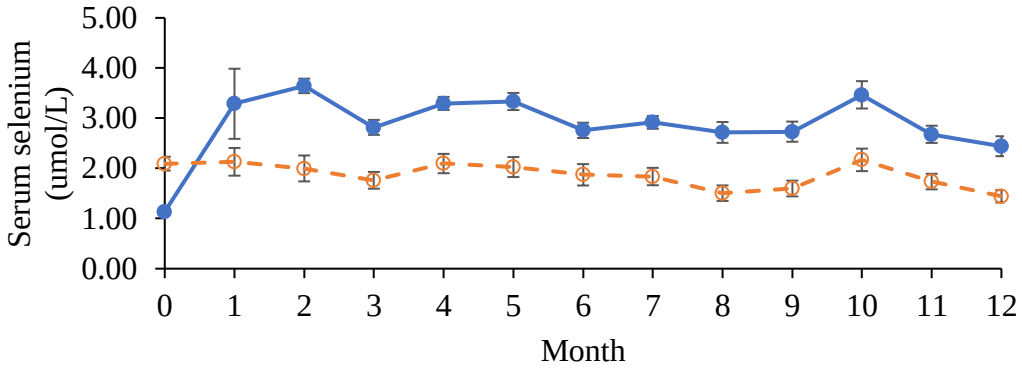

Figure 1: Monthly mean serum selenium concentrations (µmol/L ± SEM; normal range 1.50-2.60 µmol/L) for camels injected subcutaneously with 200 mg barium selenate 8 days after the first blood test (n=11; solid line) and untreated control (n=9; dashed line) camels.

Inadvertently, two 10 kg mineral salt blocks containing selenium were placed into the yard after the ninth month of testing, and this was detected when serum selenium concentrations increased in all camels at the tenth month of testing. This is depicted in Figure 1. The blocks were in the yard for approximately 25 days and it was estimated that each camel ingested approximately 0.46 mg selenium/day (11.5 mg/camel in total, or 0.03 mg/kg body weight).

Discussion

A single subcutaneous injection of 0.5 mg/kg barium selenate increased serum selenium concentrations in a group of deficient camels for approximately 12 months. Unfortunately, circumstances did not permit testing beyond 12 months, and camels were inadvertently supplemented with oral selenium during the trial, so was not possible to determine the full duration of effect of the barium selenate treatment.

Selenium deficiency has been associated with sick camels (Hassan et al., 2018) and may have an adverse effect on camel reproduction (Ali et al., 2019). Nevertheless, chronic selenium toxicity has been induced experimentally in camels fed 8-16 mg selenium as sodium selenite per day for more than 2 weeks (Faye and Seboussi, 2009) so it would be prudent in a future study to weigh camels accurately to ensure each camel only received 0.5 mg/kg barium selenate and to ensure no supplementary selenium is offered during the trial, to validate that an annual dose of 0.5 mg/kg barium selenate SC is safe and effective for Dromedary camels being fed a low selenium diet.

In the interim, annual monitoring of serum selenium concentration and supplementation of deficient camels with a subcutaneous injection of barium selenate at a dose rate of 0.5 mg/kg provides a simple, controlled method of selenium supplementation to optimise camel health and fertility.

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Venous blood lactate concentrations in healthy dromedary calves at birth and at 24 h of age

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Introduction

Due to their extreme adaptation to harsh environmental conditions dromedary camels are important livestock in arid and semi-arid regions; however, neonatal mortality is one of the main contributions to the low reproductive efficiency that affects farm profitability, in this species (Nagy and Juhász, 2019). Therefore, more studies are necessary to decrease neonatal mortality and every tool for the best evaluation and management of the newborn dromedary camel must be improved.

In many species, blood lactate plays a significant role in the assessment of the newborn, as well as in the evaluation of the critically ill patient. In human medicine, lactate in umbilical vein samples and from the scalp of the neonate is considered the best parameter to diagnose intra partum fetal hypoxia (Borruto et al., 2008). Moreover, lactate in the umbilical artery is a valuable marker of neonatal morbidity at term (Tuuli et al., 2014). In adults and neonates of many species, elevated blood lactate concentration is common during sepsis, inflammation, hypovolemia, tissue hypoperfusion and arterial hypoxemia. Hyperlactatemia, although it does not provide diagnostic information, indicates the severity of illness and the need for an early and aggressive intervention (Castagnetti et al., 2010; Borchers et al., 2013; Castagnetti et al., 2017).

In many species, blood lactate concentration is physiologically high at birth and decreases during the first hours of life (Castagnetti et al., 2010; Bonelli et al., 2015; Castagnetti et al., 2017). Therefore, the interpretation of this parameter within the clinical evaluation of the newborn must take in consideration the age in hours, especially during the first day of life. In healthy foals, blood lactate was reported to significantly decrease

to adult normal values at 24 hours of age, and only from this time-point did sick foals show significantly higher concentrations than healthy ones (Castagnetti et al., 2010).

Traditional methodologies for lactate measurement include wet chemistry analyzers or blood gas analyzers, which are typically available in large veterinary hospitals. However, several relatively inexpensive, easy to use, and rapid handheld analyzers have been validated in human and animal patients (Castagnetti et al., 2010). Although the on-site lactatemia assessment by handheld analyzers could be of utmost importance in the clinical evaluation and management of neonates under field conditions, to the best of the authors' knowledge, scarce information is available on reference ranges in dromedary newborn calves (Tharwat, 2015). The aim of this study was to investigate the venous lactate concentration in healthy dromedary newborn calves at birth and at 24 hours of age.

Materials and Methods

The study included 14 newborn dromedary calves in a free stall barn located in Al-Qassim, Saudi Arabia, born alive after spontaneous parturition and at term after a median gestation length of 377 d (min-max: 347-400 d). A venous blood sample was drawn from the jugular vein within 4 hours after birth and at 24 hours of age.

Immediately after collection, lactate concentration was measured with the handheld Lactate Scout analyzer (SensLab, GmbH, Germany). The system consists of a handheld analyzer and single-use reagent strips with a measurement range of 0.5 to 25 mmol/L. This analyzer uses an enzymatic- amperometric system for lactate detection in capillary blood. The test strip uses L-lactate oxidase to catalyze the oxidation of L-lactate (Castagnetti et al., 2010). Lactate Scout was cleaned, calibrated, and operated in accordance with the manufacturers' instructions.

For each calf, health status was recorded at 2 and 7 days of age. Time-dependent changes in blood lactate concentration were statistically evaluated with Paired Samples Wilcoxon Test.

Results

Results showed that venous lactate concentration significantly decreased from birth to 24 hours of life ($p<0.01$). An age-specific normal range for lactate concentration was calculated from data collected and is shown in Table 1.

Table 1: Median and min-max venous lactate concentration within 4 hours from birth and at 24 hours of life in healthy dromedary calves.

Sampling time	Number of samples	Median (mmol/L)	Min (mmol/L)	Max (mmol/L)
< 4 hours of life	14	4.15	1.7	7.0
24 hours of life	14	0.8	0.5	2.2

Discussion

Albeit only preliminary, the results of the present study indicated that healthy newborn dromedary calves showed a significant decrease of lactate concentration during the first 24 hours of life, as also reported in foals and bovine calves (Castagnetti et al., 2010; Bonelli et al., 2015). As in other species, the high concentrations measured at birth could be due to cortisol release or to the physiologic hypoxia during the birth process. Another cause could be the massive increase in catecholamines that occurs in the fetus during labor and helps to preserve blood flow to the brain, heart and adrenal glands and to promote post-natal adaptive circulatory changes (Tharwat, 2015).

When compared to foals' and bovine calves' concentrations (mean 3.4, range 1.7-10.2 and mean 4.8, range 1.7-9.4 mmol/L, respectively), the results showed an intermediate median concentration and similar min-max values at birth. Conversely, at 24 hours of age, the median concentration and min-max values were lower than in foals and bovine calves (mean 2.1, range 1.0-3.7 and mean 3.7, range 1.9-7.2 mmol/L, respectively) (Castagnetti et al., 2010), highlighting a possible sharper decrease of lactatemia in newborn dromedary calves. Under normal physiological conditions, the liver reconverts approximately 60–70% of plasma lactate to pyruvate while the kidneys reconvert approximately 20–30% (Pang and Boysen, 2007). Therefore, a more efficient hepatic and renal metabolism of lactate in this species, compared with equine and bovine neonates, could be the cause of a faster lactate clearance.

Our results underline that the age of the calf should be carefully considered when interpreting lactate values, especially during the first 24 hours of life.

Since lactate measurement is a simple and inexpensive tool for the diagnosis of metabolic acidosis associated with hypoxia-ischemia in many species, lactate evaluation and monitoring could also be very useful in the field for the dromedary species to help the veterinarian decide about referral, or in evaluating the response to treatment. It is also

important to underline that a whole blood gas analysis costs nearly three times more than a single lactate measurement (Pirrone et al., 2012).

In conclusion, this study provided the first recorded data about time-dependent changes in blood lactate concentration in healthy newborn dromedary calves from birth to 24 hours of age.

Further studies are needed to confirm the blood lactate reference ranges for healthy newborn dromedary calves, and to evaluate its role in diagnostic and prognostic parameters in sick ones.

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Blood gas analysis in healthy dromedary calves during the first 3 weeks of age

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Introduction

Mortality of neonatal farmed livestock within the first month of life is considered one of the major constraints that affects animal welfare and impacts farm profitability, and this also occurs in the dromedary camel species (Salih et al., 1998). Sick calves' assessment is still commonly based mainly on clinical examination; however, the emergence of pen-side blood gas analyzers has facilitated the diagnostic work-up and the assessment of degree and nature of alterations of the acid-base balance. Blood gas and electrolytes reference ranges aid clinicians and researchers in differentiating normal from abnormal parameters, and supplements the disease's diagnostic process (Knowles et al., 2000). The most appropriate reference range is generated from a group of healthy animals with physiological and environmental characteristics as closely related to the target patient as possible (Meyer and Harvey, 2004; Roland et al., 2014). In the bovine, it has been demonstrated that specific reference ranges are required from a particular age or breed of calf (Mohri et al., 2007). Therefore, the development of reference ranges of hematological parameters in dromedary camel calves of different ages would allow for more accurate identification of healthy and sick animals (Russell and Roussel, 2007; Bleul et al., 2007). Blood gas values of newborn dromedary calves were reported immediately after birth (Tharwat, 2015), but no information is available regarding the blood gases and acid-base profile in young dromedary calves. Therefore, the aim of the present study was to investigate the blood gases, electrolytes and acid-base values in healthy dromedary calves during the first three weeks of age.

Materials and Methods

Sample population

A total of twenty-one dromedary camel calves aged between one and 21 days (d) and reared in a single farm located in Al-Qassim, Saudi Arabia, were enrolled. The health status of each calf was assessed on the day of sampling through a complete physical examination performed by veterinarians, and only calves that were deemed clinically healthy were enrolled in the study. Age (d) at sampling was recorded.

Blood sampling and analysis

A venous blood sample was drawn through jugular venipuncture into 2.5 ml lithium-heparin syringes, and immediately analyzed by a VETSTAT® analyzer (IDEXX, USA). Single-use disposable cassettes were employed for the assessment of sodium (Na^+), potassium (K^+), chloride (Cl^-), hydrogen ion concentration (pH), partial pressure carbon dioxide (PCO_2), partial pressure oxygen (PO_2), total hemoglobin concentration (tHb), hemoglobin oxygen saturation (SO_2), total carbon dioxide (tCO_2), bicarbonate (HCO_3^-) and anion gap (AG) in whole blood.

Statistical analysis

Calves were divided into three groups according to their age: between 1 and 7 d of age (group A, n=8), between 8 and 14 d of age (group B, n=7) and between 15 and 21 d of age (group C, n=6). Median and min-max values for each parameter were calculated for each group, and statistical tests were applied to determine: 1) differences between groups for each parameter (Kruskal-Wallis test followed by Dwass-Steel-Critchlow-Fligner pairwise comparison); 2) correlation between parameters within each group of calves (Spearman coefficient of correlation). Significance was set for $p \leq 0.05$.

Results

Results regarding the median (min-max) values concentrations for each parameter in the three groups of calves are shown in Table 1. Significant differences among groups were found for Na^+, with higher values in group B compared to C ($p<0.05$), and for K^+, with lower values in group A compared to B ($p<0.05$) and C ($p<0.01$). In group A, the following correlations were detected: tCO_2 - BE; Cl^- - Na^+; SO_2 -BE; SO_2 -tCO_2; SO_2 - HCO_3^-; pH-PCO_2; pH-AG; PO2 -SO2; PO2 -BE; PO2 -tCO2; PO2 - HCO3$^-$. In group B, PO_2 was correlated with SO_2, and tHb was correlated with K^+. In group C, statistical analysis showed a correlation between tCO_2 - BE, SO_2 - Cl^-, and PCO_2 - pH.

Table 1: Median (min-max) concentrations for each parameter in the three groups of dromedary calves

Parameter \ Group		A	B	C
Na^+	mmol/L	**155^{ac}** (147-163)	**154^{ab}** (152-155)	**149^{c}** (147-154)
K^+	mmol/L	**4.0^{a}** (3.2-4.4)	**4.8^{b}** (4.1-5.5)	**4.6^{bc}** (4.3-5.6)
Cl^-	mmol/L	**117** (111-124)	**116** (114-119)	**113** (111-116)
BE	mmol/L	**2.30** (0.1-3.6)	**2.75** (-1.1-4.3)	**1.50** (0.0-4.2)
HCO_3^-	mmol/L	**27.1** (25.2-29.3)	**28.9** (23-31)	**27.6** (26.5-29.4)
AG	mmol/L	**13.8** (12.8-16.6)	**14.3** (11.8-16.2)	**13.8** (9.7-15.9)
pH	a.u.	**7.38** (7.35-7.4)	**7.37** (7.26-7.39)	**7.37** (7.3-7.4)
PCO_2	mmHg	**49** (48-57)	**54** (43-71)	**53** (50-61)
PO_2	mmHg	**35** (24-37)	**30** (29-82)	**33** (27-36)
SO_2	%	**66** (55-74)	**55** (55-80)	**60** (55-67)
tCO_2	mmol/L	**28.5** (26.6-31)	**30.8** (24.3-33)	**29.4** (28.1-31)
tHb	g/dL	**11.9** (11.4-12.8)	**11.6** (5.4-12.6)	**10.7** (7.5-14.4)

^{a,b,c} different superscript letters within rows indicate significant differences between groups

Discussion

Blood gas analysis combined with appropriate reference ranges for healthy animals represents a prompt and useful tool in the clinical assessment of diseased animals. The hypothesis that reference ranges for blood gases parameters should be specific for the age of target patient (Meyer and Harvey, 2004; Roland et al., 2014) was proven in other species (Rice, 1994; Herfen and Bostedt, 1999). Results of the present study partly corroborate this hypothesis, as a difference in electrolytes profile in dromedary calves of different ages was detected. In the current study, a 7-day interval was considered for grouping dromedary calves by age, according to previous results in the bovine calf (Dillane et al., 2018). Venous K^+ concentrations significantly increased across time in calves from group A to group B and C; conversely, a reduction trend of venous Na^+

median concentrations was registered from younger to older calves, with a significant decrease from group B to group C (154 *vs* 149 mmol\L, respectively).

Median concentrations of K^+ and Na^+ registered in calves 1-7 days old in the current study were similar to the mean concentrations reported by Tharwat (2015) in dromedary calves immediately after birth (4.4 mmol/L and 156 mmol/L, respectively). All the other blood gases and acid-base parameters did not change significantly among calves of different ages. This may be due to the fact that by one week of age the main respiratory and metabolic adaptations have taken place. Furthermore, due to the wide variability, a greater number of samples maybe necessary to detect a difference. The same reason might justify that some correlations between variables were not constantly identified. Noteworthy, mild subclinical and transient imbalances cannot be completely ruled out.

Although the present results cannot represent reference ranges for dromedary calves, as only a few calves were enrolled, they may be used as a guide when evaluating the blood gases, electrolytes and acid-base profile in calves of this species. This is of fundamental importance in the diagnosis and treatment of the most common diseases affecting the newborns during the first month of life, such as diarrhea and respiratory diseases.

In the present study, some efforts were adopted to avoid possible confounding factors; a single farm was enrolled, and calves were all sampled on the same day, in order to reduce farm and environmental influences on the blood gas results. However, further studies are needed on a greater number of dromedary calves, and also to evaluate the influence of other variables such as sex and breed on the blood gas profile of the dromedary calf.

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Agreement between serum Brix refractometry and IgG, γGT and total protein in dromedary camel calves

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Introduction

Camelids have an epitheliochorial placenta that prevents most of the trans-placental transfer of immunoglobulin G (IgG) to the fetus, similar to cattle, sheep and horses (El-Hatmi et al., 2006a). Consequently, ingestion and absorption of an adequate amount of immunoglobulins from colostrum is pivotal to providing antibodies which protect the newborn calf from potential infectious hazards (Azwai et al., 1996; El-Hatmi et al., 2006b). The assessment of passive transfer of immunity (PTI) is very important for the correct management of newborns, especially in the event that this process fails. Multiple tests are available to determine IgG passive transfer status of a newborn. While the radial immunodiffusion test is considered the gold standard, other tests have been suggested as alternatives, but none of them is intended for application in field practice. The indirect evaluation of IgG can be obtained through measurement of serum total protein (TP) or ɣ-glutamyl transferase (GGT). However, TP and GGT do not always directly correlate to the serum concentrations of IgG, and clinicians should use caution when using indirect concentrations as a sole predictor of adequate PTI (Johnston et al., 1997). Moreover, the assessment by laboratory analysis could be unworkable and expensive for pastoral systems, where more practical tools are advisable (Schoos et al., 2021). An effective on-farm tool should in fact be inexpensive, easy to use, and accurate. A good correlation between serum Brix score (%Bx) measured by refractometer and the IgG concentrations proved the usefulness of Brix refractometer as an accurate test for the assessment of PTI in newborn bovine calves (Deelen et al., 2014) and foals (Elsohaby et al., 2019). In order to demonstrate the usefulness of Brix refractometer for the on-farm evaluation of PTI in dromedaries, the present study aimed to assess the association

between serum %Bx and serum IgG, γGT and TP in newborn dromedary calves immediately after birth (before first suckling) and at 24 hours of age.

Materials and Methods

The study enrolled 8 healthy dromedary calves reared in a free stall barn located in the region of Al-Qassim, Saudi Arabia. Calves were born at term, after a median gestation length of 381 days (min-max: 357-388 d), from healthy dams and after spontaneous parturition. Blood samples were collected from the jugular vein into K3-EDTA glass tubes immediately after birth, before the first suckling (T0), and repeated at 24 h of age (T24). At both times of collection, samples were kept at 4°C and were delivered, within 2 hours, to the laboratory of the Department of Advanced Biotechnology and Research in Buraydah. Blood samples were centrifuged at 1000xg for 15 minutes, and two aliquots of serum were obtained. One aliquot was immediately used for evaluation by an optical Brix refractometer (Kerbl, Buchbach, Germany), which measures the refractometric index of liquids on a Brix scale. The Brix refractometer was always used by the same veterinarian, and %Bx were established by identifying a blue line on the scale. The other aliquot of each serum sample was stored at -20°C until analysis of IgG, γGT and TP, performed within 1 week. Concentrations of IgG, γGT and TP were assessed through the AU480®automated clinical chemistry analyzer (Beckman Coulter, USA).

Statistical analysis was performed to detect possible differences for each parameter between T0 and T24 through a paired samples Wilcoxon T-Test. A Spearman coefficient of correlation test was used to define a possible relationship amongst all the parameters in the two sampling times. Significance was set for $p<0.05$.

Results

The results, about the median (min-max) %Brix, IgG, ɣGT and TP at T0 and T24 in the 8 dromedary calves, are showed in Table 1.

Table 1: Median (min-max) %Brix, IgG, ɣGT and TP in dromedary camel calves (N=8), at T0 and T24

	T0	**T24**
BRIX (%)	7.0 (6.3-7.5)	8.50 (7.0-9.5)*
IgG (mg/dL)	2.40 (0.0-5.6)	764 (492-880)**
ɣGT (U/L)	15.1 (5.7-23.3)	62.8 (33.5-111)**
TP (g/dL)	4.3 (4.1-4.8)	5.6 (4.6-6.4)**

Differences for each parameter between sampling times: *p<0.05; **p<0.01

All parameters showed significant increases from T0 to T24. Moreover, at T24, %Bx was significantly correlated with γGT (r=0.73, $p<0.05$), IgG (r=0.90, $p<0.01$), and TP (r=0.96, $p<0.001$). Significant correlations were also detected at T24 between IgG and TP (r=0.96, $p<0.001$), and between TP and γGT (r=0.77, $p<0.05$).

Discussion

Median concentrations of %Bx, IgG, ɣGT and TP showed a significant increase from T0 to T24, as expected due to the colostrum intake. Data regarding IgG, ɣGT and TP were consistent with those from available literature in dromedary calves. Specifically, TP concentrations at 24 h were similar to those reported by Tarwat et al. (2015) at 12 h (5.9 g/dL, after colostrum assumption), and they were above the value of 5.0 g/Dl that is considered suggestive of adequate PTI in camels (James et al., 2022). IgG concentrations at 24 h were in agreement with data previously reported in dromedary calves (7.8 g/L; Kamber et al., 2001), while ɣGT serum concentrations at birth were similar to data from Tharwat (2015) (15±2 U/L). Data regarding serum %Brix are, to the best of the authors knowledge, completely new. The main objective of this preliminary investigation was, in fact, to verify the usefulness of the Brix refractometer as a practical tool for the on-farm assessment of PTI in newborn dromedary calves, being inexpensive and easy to use. The results showed a significant positive correlation between %Bx and TP, IgG and ɣGT at 24h after birth, consistently with data reported in bovine calves (Deelen et al., 2014) and foals (Elsohaby et al., 2019). Serum TP were also correlated with IgG and ɣGT, indicating that TP can be an indirect index of IgG consumption in the dromedary calf. From a practical perspective, the Brix refractometer was found to be easy to use, and obtained results that indicated the Brix refractometer can also be employed for the indirect evaluation of PTI in dromedary calves, although the optical Brix refractometer values may be influenced by inter-operator variability (Bielmann et al., 2010). These preliminary results demonstrated the usefulness of the Brix refractometry for the on-farm practical and fast assessment of PTI in dromedary calves. Larger number of calves enrolled and further investigations are however needed to identify the cut-off values for the diagnosis of an adequate PTI and, consequently, of failure of PTI in the dromedary calf.

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Implementation of the new milk recording scheme in dairy camels

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Introduction

Intensive camel milk production has undergone major developments during the last 15 years due to its increasing demand and a greater importance and awareness of camel milk. For instance, machine milking of dromedaries has been introduced via small-scale dairy farms in Saudi Arabia, Tunisia, Australia, Europe and the USA. It has also been implemented on large-scale dairy farms in the United Arab Emirates (Wernery et al., 2004; Hammadi et al., 2010; Nagy and Juhasz, 2016; Ayadi et al., 2018). However, little is known about how to improve milk production and to select future dairy camel herds. In fact, our search of the current literature illustrates that the genetic improvement for milk production in dairy camels is comparatively slow, and this may relate to several factors, including an extended life-span, low fertility, low numbers of offspring, delayed age at first calving, high calf mortality and abortion rates, and the unknown heritability of specific dairy traits (Ayadi, 2018). The lack of a milk recording systems is also considered to be major constraint that may impede any attempt to select future dairy animals and to develop the camel agribusiness.

Milk recording system in camels

Records of individual animals are usually associated with selection for genetic improvement (Almutairi et al., 2010). The phenotypic variations responsible for productive and reproductive traits that exist between camel breeds have yet to be fully elucidated, whilst little is known about milk recording system applications in dairy camels. According to Ayadi and Aljumaah (2015), different factors should be taken into account when implementing a new milk recording scheme in dairy camels, such as; farming systems (nomadic, settled or intensive), breed variety, milking routines, operator

training and cooperation between camel farmers, recording organizations and health care institutions (i.e., veterinary services and practitioners). Besides these considerations, it must be noted that most dairy camels are raised in small nomadic systems where farmers with poor literacy skills own the majority of the herds. Dairy camel recording systems must therefore satisfy a certain number of criteria that may include: **(i)** simplified procedures, **(ii)** recording of dairy traits that are of economic importance, **(iii)** selecting data records that are useful for management, **(iv)** data records should be time and cost efficient, and **(v)** data records should unequivocally identify the best individuals in each farm as well as identify the important genetic differences between populations at a national level.

Subsequently, guidelines for dairy camel milk recording should cover milk recording processes from the enrolment of herds for milking, through to the storage of information in a database, as well as report delivery for farmers, that will help them in decision making. Regarding the recording system itself, multiple points must be taken into consideration; **(i)** milk recording has to be carried out on all camels within the herd as well as throughout the whole lactation period, **(ii)** milk yield must be registered and expressed in kg or in litres, **(iii)** the first milk recording must be performed within 90 days of calving (according to the weaning system), **(iv)** the minimum and maximum intervals between any two tests should be 30 and 45 days respectively, **(v)** milk must be weighed on a scale with sensitivity of at least 250 g or volumetrically with calibrated measures, **(vi)** milk meters and recording jars should be approved by a member of the International Committee for Animal Recording (ICAR) organization in each country after an appropriate trial, **(vii)** milk samples, or a proportional composite sample should be obtained, to determining milk composition, at each milking time –preferably with alternate (i.e. am/pm) samples on consecutive sampling days- and these must be obtained within 24 hours of the test period, **(viii)** milk analysis must be performed within four days of recording, and **(ix)** methods for the analysis of milk components must be according to ICAR instructions. Thereby, the main steps for implementing a new milk recording scheme in dairy camels should involve; **(1)** establishing performance recording organizations, **(2)** subsidizing and rewarding the responding farmers, and **(3)** standardizing the recording procedures. These steps may be considered the fundamental keys for success.

Milk recording: an essential tool for the selection of dairy camel

Individual milk production data is of vital importance as it is the main criteria used to assess the worth of dairy animals and it informs culling and mating decisions, forming the basis for herd improvement. It is also important for properly adjusting and formulating food rations, in addition to estimating profitability. One of the most important reasons to implement good milk records is to evaluate camel udder health (somatic cell counts) and nutritional management (milk urea nitrogen).

Investigating factors that influence milk production is essential in order to efficiently introduce modern technology to dromedary camel farming systems. Several reports indicate that daily milk produced by indigenous camel breeds ranges from 4 to 18 L/head, and that milk fat content varies between 1.35 and 5.85 % (Atigui et al., 2014; Ayadi et al., 2018). The energy content of milk varies largely according to each species and breed, as well as amongst individual animals and during the different stages of lactation, making necessary the standardization of recording in practice (i.e. rationing, breeding evaluation). In order to facilitate standardized comparisons of milk yields between camels, Aljumaah et al., (2013) proposed a fat corrected milk equation at a 3% fat level:

$$FCM3\% = 0.197 \times Fat\ (\%) + 0.408.$$

In practice, the correction of camel milk according to its fat level is essential for the detection of phenotypic variations between individuals and to select future dairy camel herds.

Conclusion

Performance recording with special attention to a simplified procedure for milk recording is essential for the camel industry.

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Animal health and welfare in intensive camel dairy farming

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Advantages and disadvantages of intensification of camel milk production

Although most camels are kept in developing countries under pastoral, extensive or semi-intensive systems, well-planned intensification might potentially help the further development of the species and its integration into the food production chain (Faye, 2020). Indeed, intensive camel milk production requires a significant initial investment to build the proper infrastructure for a sufficient number of animals with enough paddock space, a regular and reliable feed supply, continuous water source, well-trained professionals and veterinary service to tend the animals. It would also need to include a milking facility unit with cooling system and a sufficient milk storage area both having a continuous electricity supply. Also, producers must have reliable access to markets to be able to sell the milk. Unfortunately, today these requirements are difficult to meet in most places where camels are kept naturally. In addition, intensive production has a substantial environmental impact compared to pasture-based systems, therefore manure management must also be taken into consideration. The concentration of animals and husbandry practices could lead to the increased emergence of non-infectious and infectious diseases and antimicrobial resistance (Gilbert el al., 2021). On the other hand, if the above conditions are available, intensive production can offer a number of advantages. Primarily, it allows the efficient and cost-effective production of high quality, raw camel milk that is suitable for further processing and meets the quality requirements of the consumers of the 21st century. At the same time, such a production also ensures that the animal health and animal welfare requirements of the species are met. This is achieved by adhering to national and international guidelines, statutory requirements and standards.

Physiological and behavioral characteristics of dromedaries that should be taken into account for intensive production

It is widely documented that camels are well adapted to arid to semi-arid conditions and harsh environment. Their upper limit of the so-called thermoneutral zone is approximately 40 ºC and healthy animals are not sensitive to "heat stress" under normal watering conditions. Camels minimize water losses with various physiological mechanisms such as adaptive hyperthermia in dehydrated camels, brain cooling, nasal heat exchange, decreased and concentrated urine excretion, way of urination and dry feces; they have high tolerance to dehydration and can rehydrate rapidly after prolonged water deprivation (Wilson, 1989). However, in hydrated animals body temperature changes within narrow limits between approximately 36 to 38 ºC. Therefore, elevated body temperature above 38.5 ºC is a sign of disease and not that of adaptive hyperthermia. The daily water requirement of camels is very low, it was calculated by Schmidt-Nielsen et al., (1956) to be 4.9 litres/100 kg body weight (bw). Based on this calculation we estimated the daily water requirement of a 620 kg lactating dromedary giving 7.2 kg of milk/day to be approximately 37 litres, while the actual consumption was 45.6 liters of water a day. In addition, camels have an efficient feed conversion, therefore the requirement of dry matter intake for maintenance is approximately 1% of bw, but a 620 kg lactating dromedary requires approximately 2% of bw dry matter intake daily for the above-mentioned daily milk production. However, under natural conditions camels spend a significant amount of time grazing, browsing and searching for feed in the desert which is not the case under intensive farming conditions.

The natural and typical behavior of the species should be taken into account when developing and managing an intensive production system. Camels are social, calm and peaceful herd animals that have a strong bond within the group and also with their offspring. The physical and visual contact with the calf is vital to maintain milk production, therefore, weaning the calf (complete physical separation) occurs at a much later stage (7 to 12 months). Frequently, camels have a reputation as being aggressive and bad-tempered, and as being difficult and dangerous to handle. However, if trained and handled properly camels can became companion animals attached to their caretakers and they can equally adapt to a non-personal, large-scale husbandry system. This is due to the supposed aptitude, high cognitive function, sophisticated mental capacity and learning ability of the species. This statement is based on our personal experience and is not supported by actual research data in this species. However, cognitive capacity in other

livestock species has been already demonstrated and is a new area of research for animal welfare and for improving livestock management (Nawroth et al., 2019).

Animal health aspects of intensive camel milk production

The underlying concept of our intensive production system is that only "*happy and healthy*" camels are able to produce good quality milk close to the maximum of their genetic potential. In order to reach this aim, a comprehensive so-called Herd Health Management program has been implemented. This program has three main elements which include an animal health and biosecurity program, an animal welfare or well-being program and a breeding and reproductive management program. These elements are not independent of each other, rather they are very much interlinked sometimes are overlapping and could not be managed successfully on their own without the other elements. The Animal health program focuses on three major areas: on infectious disease control, on milking hygiene and mastitis control and on general animal health which also includes multi-factorial or non-infectious diseases and ecto-parasite control. The most important measurable criteria to evaluate the health status of a livestock production system are the mortality and morbidity rates (OAI, 2021). At EICMP, the average annual mortality rate is 2.45%, while the average morbidity rate is 25.81%. It is important to emphasize that these figures comprise data not only from the production herd, but also from newly arrived camels in quarantine that have a higher incidence of diseases. The most frequent disease is clinical mastitis (29.7%), followed by abscess formation caused by *Corynebacterium pseudotuberculosis* (13.6%), digestive and alimentary tract problems (12.5%), reproductive disorders including abortions, perinatal mortality, post-partum problems (12.5%) and generalized infections with fever (11.7%). Injuries (8.7%), lameness (3.5%) and respiratory tract conditions (2.2%) occur less frequently and the remaining miscellaneous diseases (5.6%) include eye and ear infections, generalized oedema, tympani, foot cancer, neurological syndromes, camel pox, Trypanosomiasis, and snake bites. In the literature, there is only one more report available on diseases and mortality of intensive camel dairy farming from Saudi Arabia (Agab, 2006).

Welfare of dromedaries in intensive camel milk production

Since the introduction of the Five Freedoms of animal welfare in 1979 (FAWC, 1979), the well-being of livestock species receives more and more attention. As

mentioned earlier, the animal welfare or well-being program ("*happy and healthy*" camels) at EICMP has been an integral part of the Herd Health Management program and it is in line with OAI recommendations (OAI, 2021) that includes "good housing", "good feeding" and "good handling". The proper handling and training of animals is crucial for the entire operation and helps the camels cope with the challenges of intensive production. Good training is based on the fact that dromedaries are social or herd animals with extensive vocal and body language communication skills. With proper interpretation of these behavioral signs and positive reinforcement, they can be quickly and efficiently trained for different tasks. However, there are regular or occasional procedures such as samplings (blood, milk), treatments, washing for ecto-parasite control, nail trimming and delivery assistance when individual handling and various degrees of restraint are required. The early recognition of diseased animals both at herd and individual level is an important animal welfare issue. Camels are known to have high pain tolerance and show limited clinical signs even in serious, live threatening conditions (like intestine torsion). Therefore, the proper and continuous training of staff and the regular and frequent daily health monitoring routine are essential.

Conclusion

The intensification of the camel dairy industry started 15 to 20 years ago but such production systems are not widespread, although, it is expected to grow in the coming decades. Intensive camel milk production requires a significant initial investment, which has to include a continuous water and electricity supply, a regular and reliable feed source, as well as well-trained professionals and veterinary service, a processing facility and reliable access to markets. If the above conditions are available, dromedaries can be integrated efficiently into an intensive production environment. However, such a system requires the implementation and the strict execution of a Herd Health Management program and the compliance with statutory requirements in order to produce good quality and safe raw camel milk from "*happy and healthy*" animals. The experience at EICMP clearly demonstrates that intensive camel milk production is sustainable, a better than average animal health status can be maintained and the welfare requirements of the animals can also be fulfilled. In addition, no undesired effects associated with intensification such as the emergence of zoonotic diseases and antibiotic resistance were detected.

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Welfare of dromedary camels: what do we know?

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Introduction

Welfare is a term that describes the quality of living of an animal and animal welfare science focuses on measurable indicators that can be used to determine the welfare of animals at any given point in time. The assessment of welfare requires a multi-dimensional approach and aims to determine the actual welfare of animals, including considerations of both physical and mental states, using environmental- (EBM) and animal-based (ABM) measures. The Dromedary camel (*Camelus dromedarius*) is one of the most important natural resources in Africa and the arid lands of the Middle East and Western Asia (El Harrak et al., 2011; FAO, 2020). In these countries, the dromedary camel plays a role of great economic and social importance, as it is used for transport, racing, tourism, and perhaps most importantly provides food of high nutritional value (meat and dairy products), as well as wool and leather in a region where other common ruminant livestock species cannot be reared efficiently (El Harrak et al., 2011). However, the farming systems of these animals is shifting from pastoralism to intensive and semi-intensive breeding systems, and potential welfare concerns are rising. Currently, no comprehensive welfare standards for this species exist worldwide. Our aim in this report was to critically review the literature and present the latest updates on dromedary camel welfare.

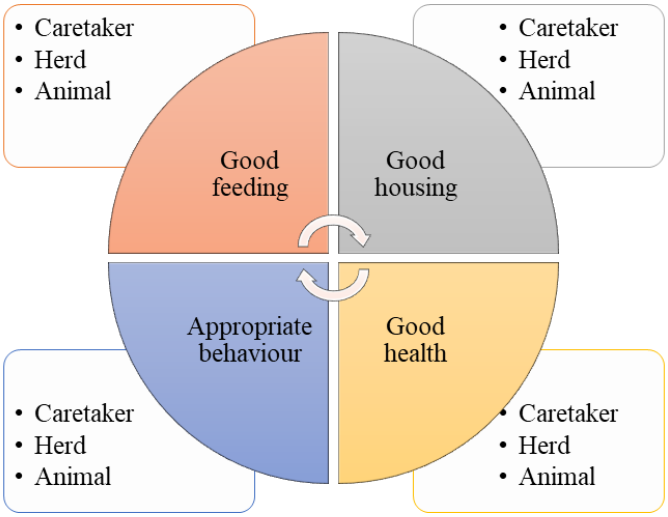
Material and Methods

The literature search was performed using PubMed, Web of Science, and Google Scholar (consulted up to April 2022). Only English language papers were considered.

Scientific tool to assess welfare on farm

To date, the only protocol that has been proposed for the assessment of welfare in dromedary camels, that applies a multi-dimensional approach was developed by Padalino and Menchetti (2021). This protocol revisited the protocols used to assess the welfare of livestock in Europe, i.e. AWIN and Welfare Quality® protocols (AWIN, 2015; Welfare Quality Network, 2009) and adapted them for use in dromedary camels. It therefore foresees the on-farm collection of both ABMs and EBMs relating to the four fundamental welfare principles (Good feeding, Good housing, Good health, and Appropriate behaviour). However, it was inspired by Mellor's recommendations (Mellor et al., 2020) and as such, includes indicators of positive states and human-animal relationships. For each welfare principle, ABMs and EBMs are collected at three levels of assessment: Caretaker, Herd, and Animal (Figure 1), for a total of 105 measures.

Figure 1: An example of the data collection of the protocol for camel welfare assessment proposed by Padalino and Menchetti (2021). Modified by Padalino and Menchetti (2021) and AWIN (2015).



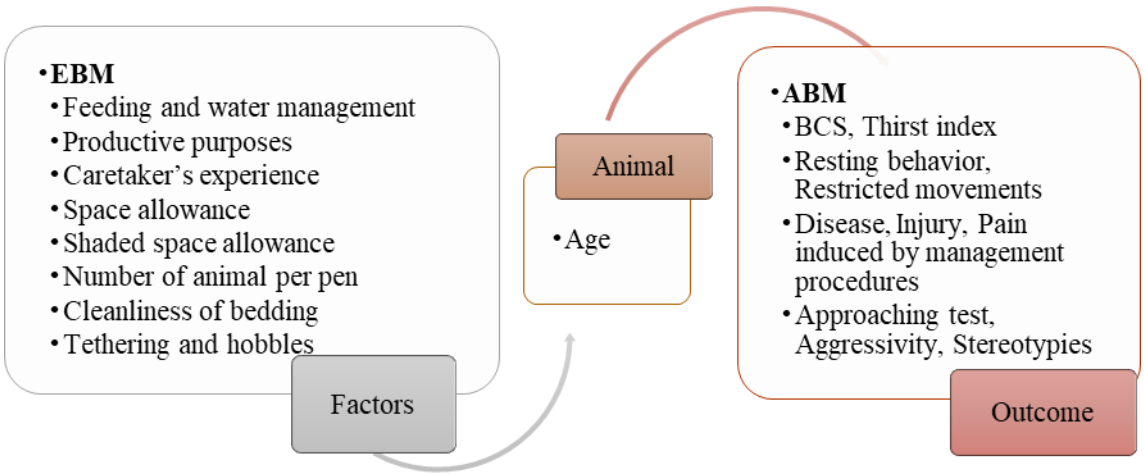
Applications on a market

Padalino's method has so far only been applied to evaluate the welfare of dromedary camels kept at a market in Qatar (Menchetti et al., 2021a, 2021b). A questionnaire was filled out by 49 caretakers; collecting information related to their background and how they manage the camels. A total of 76 pens holding 528 camels were evaluated; pen dimensions and the presence of shelter or other equipment (water and feeding points) were recorded. The body condition as well as health, and behavioural

parameters were also recorded from 2 animals randomly selected from each pen (n=132). Models used to calculate overall welfare indices and to classify pens were developed (Menchetti et al., 2021b). The evaluated parameters were scored using a 0–2 scale, and scores were aggregated through a 4-step process to obtain overall assessment indices. Based on these overall indices, most of the pens were classified as "unsatisfactory" (61.8%) and none as "excellent". Using this type of classification, it was possible to identify welfare issues and give recommendations to improve management and consequently camel welfare. Some of the recommendations have already been put in place in Doha.

Another way to identify welfare consequences and their hazards, in order to formulate preventive and corrective measures, is to apply the risk analysis in animal welfare as suggested by EFSA (2012). Data that were collected at the market in Doha were used to develop statistical models and identify possible associations between ABMs and EBMs for risk characterization (Figure 2) (Menchetti et al. (2021a)).

Figure 2: Selected environmental- (EBM) and animal-based (ABM) measures from Padalino's protocol for the characterization of risk analysis for camel welfare according to the EFSA approach (2012). Modified by Menchetti et al. (2021b). BCS= Body Condition Score.



Space allowance, the presence of shaded space, cleanliness of bedding and water management were the major welfare hazards identified for dromedary camels kept on the market. Consequently, a minimum space allowance of at least 19 m^2/camel and the provision of adequate shaded areas, were recommended in order to prevent heat stress and enhance dromedary camel welfare in this setting (Menchetti et al., 2021a).

Welfare concerns and gaps of knowledge

Interest in camel breeding has grown recently and this has led to an increase in research regarding camel health and breeding practices (Faraz et al., 2021; Hussen and Al-Sukruwah, 2022; Pastrana et al., 2021; Zappaterra et al., 2021). However, properly defined welfare standards for this species still seem a long way off. The method proposed by Padalino and Menchetti (2021) was the first attempt to develop a tool for camel welfare assessment in line with the protocols used for other livestock species, but it is applicable only for dromedary camels kept in semi-intensive and intensive farming scenarios, and the tool still requires much refinement and must undergo a thorough validation process. Even within intensive systems, the fine tuning of specific protocols would indeed be necessary for assessing the welfare of dromedary camels bred for specific purposes, such as dairy farming and racing. Dioli (2022) recently reviewed the major welfare concerns for camels reared under a variety of systems and emphasized the need to contextualize the assessment of animal welfare in pastoral areas, taking into account the unique environmental, cultural, ecological and economic settings. Despite these advances, to date, there exists no specific recommendations concerning the welfare of farmed dromedary camels within European legislation (Previti et al., 2016). Furthermore, it is important to highlight that there are still no guidelines for the welfare of dromedary camels during transport and slaughter. In conclusion, the development and application of protocols for the assessment of dromedary camel welfare from 'farm to fork' and from bird to death, within environmental and farming contexts is therefore urgently needed.

Conclusions

Overall, the number of studies on dromedary camel welfare remains limited and more research is urgently required in order to suggest welfare standards for this species.

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Camel breeding in Doha market: a survey of the caretakers

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Introduction

Dromedary camels (Camelus dromedarius) have a prominent economic and social role in several countries (Ali et al., 2018). Studies addressing management needs and reproductive behaviours of this animal species have greatly increased in recent years (Padalino et al., 2015), as well as the use of advanced biotechnologies in camel breeding (Monaco et al., 2015). However, in many camel farms and markets, husbandry and reproduction are still based on a father-son tradition, natural mating is very common, and pregnancy diagnosis is rarely conducted using ultrasound. All these features may affect the reproductive efficiency in camels (Ali et al., 2018). This study documented the background of the camel caretakers in a market (Doha, Qatar) and their breeding practices. Possible associations amongst those factors were also investigated.

Materials and Methods

The study was carried out at the Doha dromedary camel market in Qatar from the 11th to the 18th of September 2019 with the permission of the Department for Agriculture Affairs and Fisheries of the Ministry of Municipality and Environment of the State of Qatar. The target population of the survey was workers taking care of the camels housed in 92 pens. All the caretakers working at the Doha market in that period (N=49) were interviewed. During the study, 76 out of the 92 pens were occupied by animals, for a total population of 528 camels being managed by the 49 caretakers. The latter were approached by a native Arabic speaker (Dr. Ziani) and were asked for voluntary consent to take part anonymously in the research project. The Survey was designed following Dean (2015) and Christley (2016) recommendations for valid questionnaire designs. The survey consisted of 29 closed and 3 open-ended questions. The survey elicited information

related to their background (country of origin, age, education and experience level, for a total of 10 questions) and how they manage the camels, including their reproduction (2 closed and 1 open-ended questions). In particular, an open-ended question was sought to elicit information concerning how they diagnosed whether a recently mated female camel is pregnant. The answers were analysed using data management in Excel and statistical tests in R environment. The answers were categorized into classes and then used to perform descriptive statistics. The Chi-square goodness of fit test was used to compare the observed distributions of answers with the expected probability distributions. Generalized Linear Models (GLMs) were used to investigate whether demographic features, productive purposes and management choices of the caretakers were associated with different camel management and breeding practices. Associations were expressed and odds ratio (OR), 95% confidence interval (95%CI), and Wald test P-values. For all the statistical tests, a P-value <0.05 was considered statistically significant. The answers obtained from the open-ended question about pregnancy diagnosis were submitted to word cloud analysis using WordArt online tool.

Results and Discussion

All the caretakers were male, mainly from Sudan (44/48; 92%; P<0.001), young (20-30 years: 14/49, 28.6%; 31-40 years: 17/49, 34.7%; P<0.001) and with a low education level (no school:19/49, 38.8%; elementary school:12/49, 24.5%; P<0.001). Nearly all of the respondents had been working at the Doha market for more than one year (44/48; 92%; P<0.001) and had previous experiences with animal husbandry (47/49; 95.9%; P<0.001). Among them, 27 had previous experience with breeding livestock species other than camels, mainly sheep (21/27; 77.8%, P<0.001) and cows (19/27; 70.4%, P<0.001). In addition, the respondents said they had many years of experience in camel handling (more than 10 years of experience: 30/49; 61.2%; P <0.001). Most of them said they learned how to handle camels by father-son tradition or from friends and relatives (39/49; 79.6%; P<0.001), but only a few learned it during their present job experience (9/49; 18.4%). Usually (42/49; 85.7%; P<0.001) a number of camels between 6 and 30 were handled by the same caretaker, managing 1 (19/49; 38.8%), 2 (24/49; 49.0%), or more than 2 (6/49; 12.2%) different pens. The camels were kept at the market for different purposes: only for slaughter (5/49; 10.2%), as live (considering camels kept for milk, breeding and race, 25/49, 51.0%), or for both utilisations (19/49; 38.8%,

P=0.002). Only 33 caretakers reported taking care of she-camels and managing their reproduction. Only natural mating was practised, with the possibility to choose the bull. In only one pen, was there a bull free in the herd. There was no association between the years of experience of the caretakers and reproductive management (P=0.816), but usually, the caretaker chose the time for mating (23/33; 69.7%; P <0.001). In about half of the cases, the owner intervened in the choice of the bull (16/33, 48.5%). However, in the cases where the caretaker chose the time for mating, he would be more likely to choose the bull (OR:16.9, 95% CI:2.5-341.2; P=0.013). Approximately two weeks after mating, the majority of the caretakers (32/33, 97.0%) exposed the she-camel to a bull, monitoring her behavioural reactions. Only 19/32 caretakers gave further details, reporting that they considered the female pregnant if she tried to escape from the male (2/19; 10.5%), lifted her tail (17/19; 89.5%, P<0.001) or urinated (1/19; 5.3%) (Figure 1).

Figure 1: Word cloud with the responses to the open-ended question "How do you check pregnancy?". Larger words were the most used by the respondents.

Despite the fact that most of the respondents answered that they would regularly call a veterinarian to check the health of the camels (30/47; 63.8%, P<0.001), or if a camel was sick (43/49; 87.8%, P<0.001), none of them responded that they would call a veterinarian to diagnose pregnancy in the mated females. This seems to suggest that while the role of

the veterinarian is considered to be pertinent as regards the evaluation of the animal's health and the treatment of pathological states, the management of reproduction is still almost exclusively the role of the caretaker. In several studies and husbandry guidelines, tail lifting (or cocking) in pregnant females exposed to bulls has been indicated as an early marker of pregnancy (Rathore, 1986; Moretti, 2009). However, this behaviour in some cases may be inhibited, in particular when the mated female is under stress (Skidmore, 2000). Thus, the observation of females' behaviour after exposure to a bull may be more accurate when combined with other more accurate tools, such as ultrasonographic imaging, which was proved to be effective in identifying pregnant she-camels as early as day 20 post-mating (Vyas et al., 2004). An early and accurate diagnosis of pregnancy is indeed essential to increase reproductive efficiency in this animal species (Ali et al., 2018).

Conclusions

Overall, the owners preferred to make reproduction-related decisions, such as choosing the bull, and no associations with the caretaker's background were found. Natural mating and monitoring of female behaviour to identify pregnancy were common practices among the camels' caretakers in the Doha market. While the role of veterinarians is taken into consideration in the case of sick animals, they are much less involved in the management of reproduction practices. Specific training of the camel handlers and veterinary services should be implemented for introducing modern dromedary camel reproductive management practices in camel markets and farms, which may help improve the camels' reproductive efficiency, health and welfare.

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Dromedary camel urine limits proliferation and modifies cell morphology in human renal tumoral and normal cells

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Introduction

The ethnomedical practice of consuming dromedary urine, either alone or mixed with milk, as a remedy against various illnesses is well recognized within Islamic prophetic medicine. Historical records dating back to 1020 at 'The Canon of Medicine' by Avicenna constitute the most antique registers of this practice. At the contemporaneous scene, multiple researchers have tried to unravel the bioactive potential of dromedary urine so that scientific evidence can support its therapeutical use and aid the development of new drug targets. Anti-tumoral activity, regulation of platelet aggregation, gastric epithelia protection, anticlastogenic activity and treatment of infectious processes are the principal recognized effects of this animal-origin product (Alkhamees and Alsanad, 2017; Anwar et al., 2021). In the present research, we have investigated the *in vitro* antiproliferative role of dromedary camel urine towards human renal tumoral and normal cells, as well as the changes produced in the cell morphology. To the best of our knowledge, these cell lines have not yet been considered in the available related literature.

Materials and Methods

Animal sample

Urine samples were collected from ten dromedary camels (seven males and three females; within a wide range of age and physiological status) (Table 1) reared in Doñana

National Park (southwestern Spain). The animals cohabit in the same farm and are fed on alfalfa, beet pulp, calcium carbonate, salts and selenium.

Table 1: Categorical description of animal samples.

ID	Gender	Age (years)	Physiological status
1	Female	19	Not neutered
2	Male	14	Bull
3	Male	28	Neutered
4	Male	30	Neutered
5	Male	20	Neutered
6	Female	35	Not neutered
7	Male	32	Neutered
8	Male	1.5	Bull
9	Female	4	Pregnant
10	Male	4	Bull

Urine collection and storage

Mid-stream, first-morning-void urine was collected from each animal when naturally peeing in a sterile bag placed on a cone holder. Immediately after collection, urine was centrifuged at 2,500 × g for 5 min at 4 ºC. The supernatant was then recovered and sterilized using a 0.22 μm filter. For safe long-term storage until the *in vitro* bioactivity experiments, the urine pre-processed samples were stored at -80ºC.

Cell cultures

The human proximal tubule epithelial cell line (HK2) and the human renal carcinoma cell line (Caki-1) were kindly provided by Prof. Ciro Leonardo Pierri (ORCID: 0000-0003-1816-548X). Both cell lines were cultured in high glucose Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum, in 25 cm^2 culture flasks and were maintained at 37°C and 5% CO_2.

Adjustment of urine osmolarity prior to in vitro testing

Considering the tolerance limit of the cultured cells to media osmolarity (Buchmaier et al., 2013), urine samples were diluted in different amount of culture medium based on their initial osmolarity, in order not to exceed a final value of 500 mOsm/liter.

Cell viability assay

Urine toxicity was assessed via a resazurin-based cell viability assay, that is based on the ability of living cells to reduce the oxidized non-fluorescent blue resazurin to a red fluorescent dye (resorufin) through mitochondrial reductase activity. Cells were seeded in 96 multi-well plates at a density of 15,000 cells/well in the growth medium and allowed to attach overnight; then HK2 and Caki-1 cells were exposed to urine-added medium for 24, 48, and 72 hours, at 37ºC. At each time point, the urine-added medium was discarded and 100 µl of resazurin reaction solution (Biotium®) were added into each well and incubation was then extended for 90 minutes. Fluorescence was recorded at $\lambda_{Ex/Em}$ 535/590 nm by a FLUOstar® Omega microplate reader. Experiments were performed in triplicates.

Optical microscopy analysis

The HK2 and Caki-1 cells plated and treated with dromedary urine-added medium were monitored via optical microscopy analysis, acquiring brightfield images with 10× magnification at each considered time point in order to assess cell morphology evolution over time.

Statistical analysis

Given the fact that the experimental data satisfied the normality assumption, the antiproliferative effect of dromedary urine solutions was determined using one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* tests using the SPSS statistical package. The Dunnett's test is a multiple-comparison method that searches for significant differences between the mean of several experimental groups and the mean of the control group.

Results and discussion

Dromedary urine limits the proliferation of human renal tumoral cells and modifies cell morphology of renal normal cells

When assessing resazurin-based Caki-1 viability, after 24-, 48- and 72-hours exposure to urine-added medium, a toxic effect was observed in only two, out of ten samples, with both coming from male dromedaries. Notably, urine samples from dromedaries 8 and 4 were significantly ($p < 0.05$) associated to a cell viability decline compared to controls at 24 h (8) and 72 h (8 and 4). In line with the main literature (Mahboub et al., 2014; Al-Yousef et al., 2012), we observed significant antiproliferative effects for cancer cell lines, while only marginal effects were detected on normal cell lines. Optical microscopy

highlighted clear changes in the morphology of healthy cells, but not of the tumoral ones. To the best of our knowledge, this is the first report documenting cell morphological modifications after *in vitro* exposure to dromedary urine. The above-mentioned changes were observed for six, out of ten, urine samples (from both male and female animals) and were characterized by a strong and time dependent HK2 elongation (Figure 1). Future work should therefore focus on the implication of dromedary urine (or their small molecules) in the cytoskeleton reorganization, which is known as the main element involved in cell shape determination.

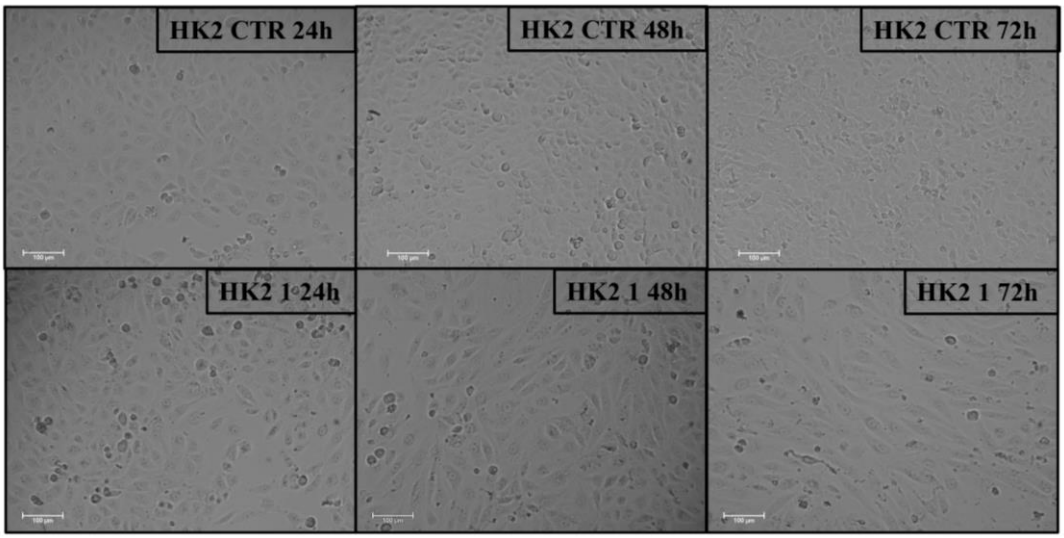

Figure 1: Time-dependent elongation in HK2 cells when exposed to dromedary urine *in vitro*. The bottom panel of images are representative of the 1 urine sample effect on the HK2 cell morphology, compared to the control condition (top panel).

Conclusions

Taken together, our results indicate that dromedary urine effects on cell viability and morphology are animal-specific, and they depend on the target cytotype. A deeper understanding of the mechanisms underlying the observed variability could be provided through metabolomic-based identification of dromedary urine bioactive compounds possibly responsible for the above observations.

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2-PBA, a small molecule observed in dromedary urine, induces morphological changes in secondary human renal cell lines

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Introduction

Natural products from plants and animals have been used since ancient times for the treatment of many diseases, and bioactive molecules of natural origin continue to enter clinical trials and provide lead compounds, mainly to develop antitumoral, anti-inflammatory or antimicrobic agents. Isolation of drug-like compounds from biological matrices, as also their downstream targets screening, remains challenging, although the technological advances in metabolomics are significantly facilitating the unraveling of this complexity (Harvey et al., 2015; Dias et al., 2012). A well-known ethnomedical practice in traditional dromedary countries concerns the use of dromedary urine as a remedy against various illnesses, via systemic or topical routes. Aiming at a deeper biological understanding of the health-related effects of this natural product, we selected three small molecules, namely 2-phenylbutyric acid (2-PBA), 3-phenyllactic acid (PLA), and oleic acid (OA), for *in vitro* bioactivity evaluation performed on two human renal cell lines (HK2, healthy; Caki-1, tumoral). These molecules were found in dromedary urine by either our ongoing NMR metabolomic profiling study or they had been described in the literature (Antakly, 2012; Ahamad et al., 2017). The most interesting results were obtained when using 2-PBA, a short-chain fatty acid *in silico* predicted to have anticancer properties, and they pointed to clear morphological changes in both cell lines.

Materials and Methods

Cell cultures

The human proximal tubule epithelial cell line (HK2) and the human renal carcinoma cell line (Caki-1) were both cultured in High glucose Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum, in 25 cm^2 culture flasks, at 37°C and 5% CO_2. Cells were regularly passaged on reaching 90% confluency with 0.25% trypsin-EDTA.

Preparation of molecules for in vitro testing

3-phenyllactic acid (PLA) and 2-phenylbutyric acid (2-PBA) were dissolved in the cell culture medium to obtain a 50 mM stock solution, which was sterilized using a 0.22µm syringe filter. Oleic acid (OA) was dissolved in DMSO to obtain a 300 mM stock solution.

Resazurin-based cell viability assay

After seeding in 96-multiwell plates at a density of 15,000 cells/well in supplemented DMEM, HK2 and Caki-1 cells were allowed to attach overnight. The next day, cells were incubated with different concentrations of molecules, selected according to a literature review, diluted in culture medium (50, 10, 1, 0.1 mM for PLA and 2-PBA; 3, 0.3, 0.1, 0.03 mM for OA) for 24, 48 and 72 hours at 37ºC. At each respective time point, the resazurin cell viability assay was performed following manufacturer's instructions. This assay detects cell viability by converting a nonfluorescent dye into the highly red fluorescent dye resorufin due to the mitochondrial activity of cells.

Optical microscopy analysis

The HK2 and Caki-1 cells plated and treated with different concentrations of molecules were monitored via optical microscopy analysis, acquiring brightfield images with 100× total magnification at 24, 48 and 72 hours to evaluate cell morphology evolution over time.

Statistical analysis

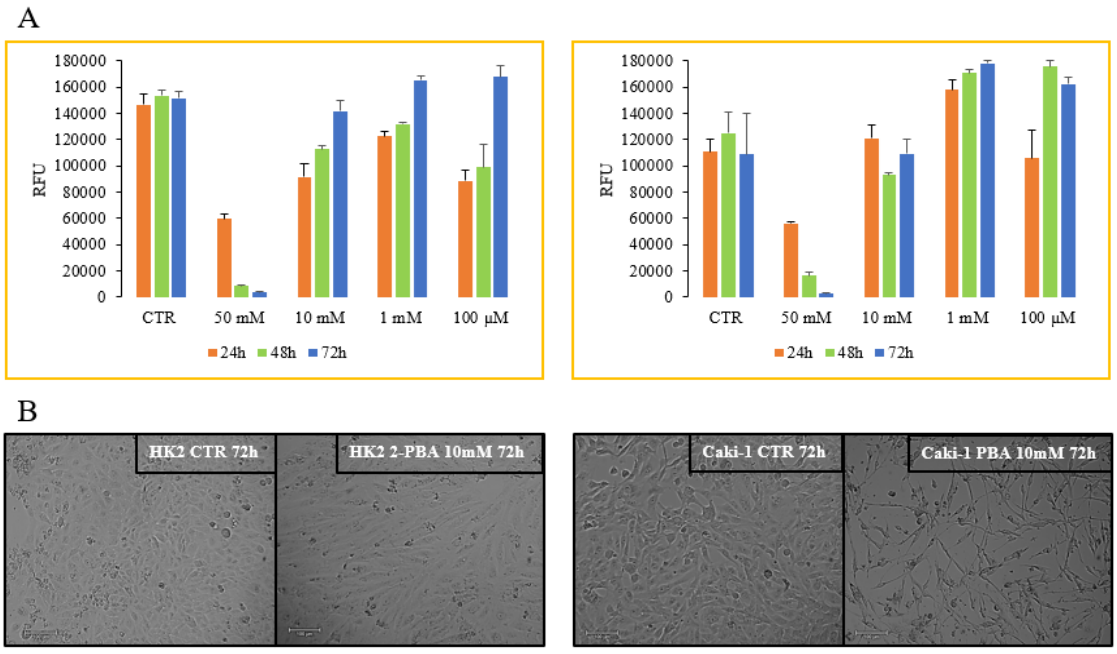
As the experimental data satisfied the normality assumption, molecules effect on cell viability was determined with a one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test using SPSS.

Results and discussion

The resazurin-based cell viability assay highlighted for PLA a strong cytotoxic effect on both cell lines when used at the highest concentration of 50 mM, and a weaker effect on cell viability at 10, 1 and 0.1 mM (data not shown); a slight elongation of HK2 cells was observed only at 10 mM. Similarly, OA induced the death of both HK2 and Caki-1 cells at the highest concentration (3 mM), while, in this case, the lower concentrations seemed to increase the viability of Caki-1 (data not shown). This is consistent with a previous report on another human tumoral renal cell line, the 786-O cell (Liu et al., 2013). No morphological changes were observed for any of the two cell lines when treated with OA.

A stronger effect on both cell morphology and cell viability was observed on HK2 and Caki-1 cells when they were treated with 2-PBA. The highest concentration (50 mM) was found to be cytotoxic in both cell lines, while the lower concentrations showed a time-dependent positive trend in cell viability for Caki-1 (Figure 1A). Remarkably, at 10 mM concentration, we observed a marked effect on the morphology of both the renal cell lines. In particular, healthy HK2 cells underwent a clear cell elongation compared to the original cobblestone shape control cells, while tumoral Caki-1 cells appeared thinner and spindle-shaped (Figure 1B).

Figure 1: A) Cell viability after treatment of HK2 (left panel) and Caki-1 (right panel) cell lines with different concentrations of 2-phenylbutyric acid. **B)** Morphological changes occurred at 10 mM of 2-PBA compared to the control condition (CTR).



In the literature, a morphological change was reported for liver carcinoma cell lines treated with 4-PBA (Meng et al., 2005). The 4-PBA is a particularly active molecule, known as a histone deacetylase inhibitor, ammonia scavenger, ER stress inhibitor and chemical chaperon, with neuroprotective and anti-inflammatory effects (He and Moreau, 2019). According to our preliminary analysis of ChEMBL and Kegg-ligand bioactive molecules databases, 2-PBA is likely to target the same proteins targeted by 4-PBA.

Conclusions

Our *in vitro* study highlighted the ability of 2-PBA, a small molecule identified through a preliminary metabolomic analysis of dromedary urine, of inducing relevant cell type-specific morphology modifications, together with various extents of reduction in cell viability. Further investigations are needed to better understand 2-PBA bioactivity and the underlying molecular processes.

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New genomic tools in Old and New World camelids for assessing reproductive and other phenotypic traits

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Large and small camelids represent a key livestock resource in many countries. Due to their unique assortment of biological and physiological traits and their adaptations to extreme and harsh conditions, camelids can thrive and produce high-quality food and fibre (wool) products in environments where other livestock species struggle. Recently, chromosome-assembled reference genomes of two Old World camel species, the dromedary (*Camelus dromedarius*) and the wild two-humped camel (*Camelus ferus*), as well as two domesticated New World camel species, the alpaca (*Vicuna pacos*) and llama (*Lama glama*), have become available. However, a high-resolution radiation hybrid (RH) chromosome map of the camel genome is still missing, as well as an affordable and easily applicable DNA microarray tool for large-scale phenotype-genotype association studies for traits of interest.

In the current study, we designed a 180K Affymetrix-Axiom (Thermo Fisher Scientific) custom array for camelids, including 60K single nucleotide polymorphisms (SNPs) for dromedaries, 60K SNPs for two-humped camels (Bactrian camel and wild camel) and 60K SNPs for New World camels (llama and alpaca). Furthermore, 11K SNPs were included at locations within known immune response genes, that may be used in order to study associations with emerging pathogens. This array is currently under evaluation at the Animal Production and Health (APH) Laboratory of the Joint Food and

Agriculture Organisation (FAO)/ International Atomic Energy Agency (IAEA) Division, Austria. This array will be used to (i) genotype the 5000 RAD dromedary RH panel previously constructed at the APH laboratory, in order to create a high-resolution RH map of the dromedary genome, and (ii) to perform diversity and association studies in camelids to better understand the genetic basis of interesting reproduction, milk, meat, and health traits as well as to identify local adaptations. In addition, more camelid genomes sequenced at high coverage are on their way. With these new genomic resources, we are a step closer to a sustainable utilisation of Old and New World camels in their challenging environments.

Validation of the camelid SNP array for dromedaries

As a first step of validation, the array was tested on a panel of 96 dromedary camel samples in order to generate library files that can convert signal data into genotypes. The process of validation of the 60K dromedary SNP was successful with extraction of genotypes at ~86% of loci under highly stringent thresholds and quality control parameters (DQC> 0.82, SNP QC call rate >97% , the average call rate for passing samples was ≥ 98.5 and the percentage of passing samples ≥ 95). The array consisted of 62707 probe sets covering 59958 genome wide SNP markers specific to dromedary camels (Figure 1). Of these, a total of ~51500 markers were successfully genotyped that included ~44860 PolyHigh Resolution (presence of both homozygotes and heterozygotes), 4660 NoMinor Homozygotes (absence of minor allele homozygotes) and ~2360 MonoHigh Resolution (monomorphic) SNPs (Figure 2).

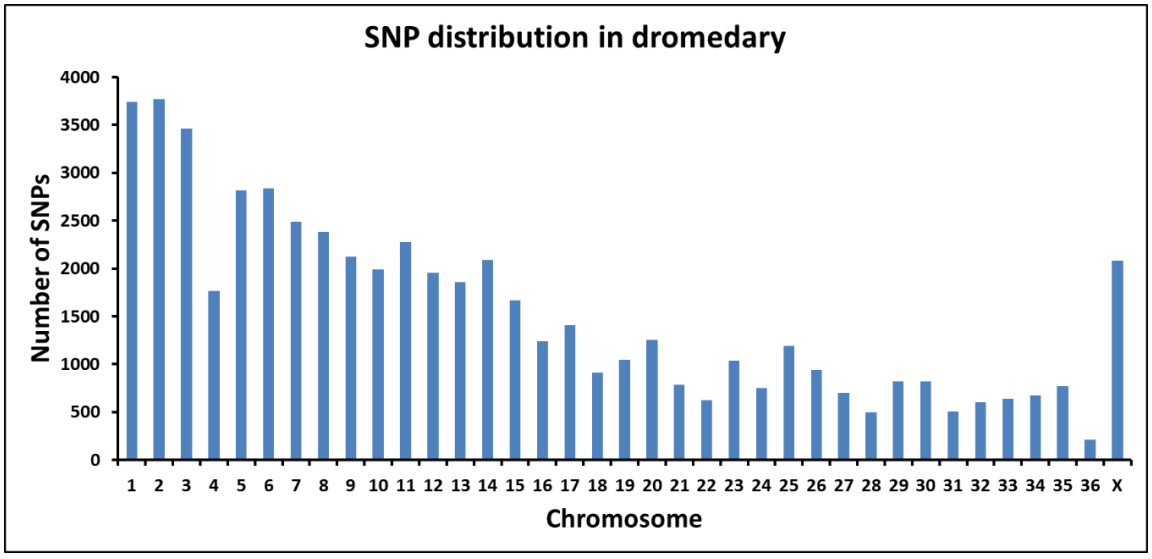

Figure 1: Chromosomal distribution of dromedary SNPs in the multi-species camelid array.

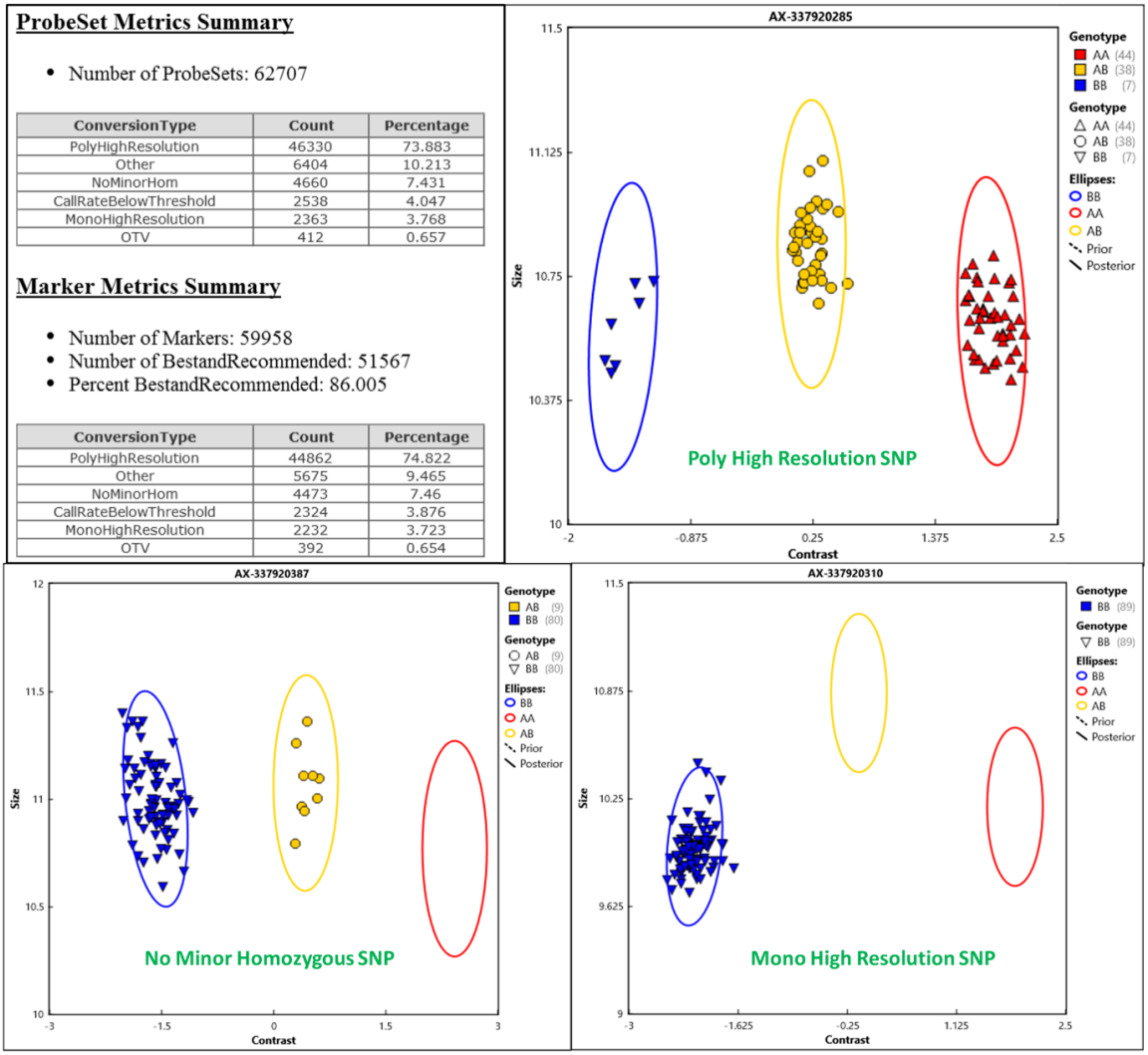

Figure 2: Example SNP cluster plots (Poly High Resolution, No Minor Homozygous, and Mono High Resolution) for dromedaries.

Testing and validation of the Bactrian camel SNP panel in the multi-species camelid array

To validate the 60K Bactrian camel SNP panel, 96 samples (Chinese and Mongolian Bactrian camel) were tested to generate library files that could convert signal data into genotypes. The validation process was successful with the extraction of genotypes at more than 51000 marker loci with a success rate of 86.2%. The thresholds for quality control parameters were set high with DQC>0.82, SNP QC call rates >97%, the average call rate for passing samples ≥ 98.5% and the percentage of passing samples ≥ 95%. About 67.32% of genotyped markers were classified under the PolyHigh Resolution category (presence of both homozygotes and heterozygotes), 10.52% under the NoMinor Homozygotes category (absence of minor allele homozygotes) and 8.36% under the MonoHigh Resolution (monomorphic) category. When dromedary specific

library files were used to genotype Bactrian camel samples, ~79.8% of markers were successful, of which 6.7% were classified under the PolyHigh Resolution category and 37% under the NoMinor Homozygotes category. This approach can help in generating data on 25K additional markers for Bactrian camels.

Validation of the New World camelid SNP panel in the multi-species camelid microarray

In order to validate the 60K New World camelid SNP panel, 280 samples collected from four different New World camelid species (alpaca, llama, vicugna and guanaco) and were analysed on the array. The raw signals were utilized to generate genotyping library files, specific for New World camelids. The validation process was successful with the extraction of genotypes at more than 53000 marker loci and a success rate of 88.47%. The thresholds for quality control parameters were set high with DQC>0.82, SNP QC call rates >97%, with the average call rate for passing samples ≥ 98.5 and the percentage of passing samples ≥ 95. About 30,400 (~50.7%) markers were classified under the PolyHigh Resolution (presence of both homozygotes and heterozygotes) category, ~14100 markers (23.64%) under the NoMinor Homozygotes (absence of minor allele homozygotes) category and ~8400 (~14%) under the MonoHigh Resolution (monomorphic) category. This successful validation has now enabled genetic and genome wide evaluation of New World camelid species.

Refining the *Camelus dromedarius* myostatin gene polymorphism through world-wide whole-genome sequencing

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Introduction

Myostatin (*MSTN*), also known as Growth Differentiation Factor-8 (*GDF8*), is a highly conserved negative regulator of embryonic development and homeostasis of skeletal muscle in mammals. This gene has been largely studied in several livestock and model species where many functional mutations have been described, with some of them being known to produce a hyper-muscular phenotype called "double muscling". In *Camelus dromedarius*, the gene structure organization and the sequence polymorphisms have been previously investigated using Sanger (Muzzachi et al., 2015) and Next-Generation Sequencing (Favia et al., 2019) technologies on a limited number of animals. Here, with reference to the myostatin locus, we present the results of a Next-Generation Sequencing project involving 161 samples from 18 countries complemented with publicly available whole-genome sequences from 22 additional animals.

Materials and Methods

We carried out a follow-up study with the aim of deepening our knowledge on the sequence polymorphisms at the myostatin locus previously investigated by Muzzachi et

al. (2015) and Favia et al. (2019). This research is part of the "2019 Agricultural Greater Good Initiative", an Illumina®-funded project. A total of 161 dromedary biological samples (whole blood/hair follicles) were collected through a large international collaborative effort. Genomic DNA isolated from these samples was whole-genome sequenced using the Illumina NovaSeq System (Illumina, CA, USA). In addition, we also capitalized on 22 dromedary, publicly available, whole-genome sequence datasets, for a total of 183 samples representative of the geographical distribution range of this species (Table 1). We restricted our polymorphism analysis to the ± 5kb upstream and downstream region of the *MSTN* gene. The identified variants were classified based on their nature, localization and occurrence in the previously published paper by Favia et al. (2019). Subsequently, an inter-specific comparative analysis was carried out by BLASTing the *C. dromedarius* myostatin sequence (GCA_000803125.3) against the corresponding sequences in *C. ferus* (GCA_009834535.1) and *C. bactrianus* (GCA_000767855.1).

Results

In the 16,757 bps considered region we identified 99 variants, out of which 77 were Single Nucleotide Polymorphisms (SNPs) and 22 were indels. Based on their localization, 72 variants were intergenic (namely, 38 variants in the 5 kb region upstream the *MSTN* gene and 34 variants in the 5 kb region downstream the *MSTN* gene), 1 SNP in the 5' UTR region, 1 SNP in exon 1, 11 variants in intron 1, 1 SNP in exon 2, 9 variants in intron 2, 4 SNPs in exon 3. Moreover, the 11 SNPs previously identified by Favia et al. (2019) in the sequence overlapping with our target region, were also found in our 183 samples dataset. Interestingly, none of the 6 exonic SNPs resulted in a missense mutation. The inter-specific comparative BLAST analysis showed that *C. dromedarius* is the most divergent species, as also supported by previous studies within the *Camelus* genus (Wu H. et al., 2014, Geraads et al., 2020).

Discussion

An average density of one SNP about every 217 nucleotides was observed in this study. This higher density compared to what was observed by Favia et al. (2019), i.e. one SNP about every 1.5 kbps, may derive from the larger and more geographically representative population sample-set available from the Illumina®-funded "2019 Agricultural Greater Good Initiative." We also detected here, for the first time, SNPs

within the exonic regions of the myostatin (n= 6). However, none of them caused amino-acid substitution at the protein level. Also, out of the 22 indels detected in the target region, none of them was located in the exonic regions of the myostatin gene. We are currently exploring potential functional effects, such as differential transcription factors binding and altered splicing mechanisms, for the detected polymorphisms through *in silico* prediction. Neutrality tests also represent a further analytical goal, as the observed sequence variation patterns seem to point to a possible evolutionary constraint in this species.

Acknowledgements

We want to thank all the project partners and collaborators who provided biological material for this study.

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Table 1: Details about the adopted population sample-set and the publicly available whole-genome sequence data used in this study.

Country of origin	Sample ID	Coverage (Gb)	Country of origin	Sample ID	Coverage (Gb)	Country of origin	Sample ID	Coverage (Gb)	Country of origin	Sample ID	Coverage (Gb)
Algeria	VV_Drom1359_2	86.36	Kenya	VV_Drom19	23.1	Morocco	VV_Drom1762	30.79	Sudan	VV_Drom1810	21.2
Algeria	VV_Drom1362	20.83	Kenya	VV_Drom2	23.66	Morocco	VV_Drom1764	34.93	Sudan	VV_Drom1815	24.04
Algeria	VV_Drom1363	32.09	Kenya	VV_Drom20	28.7	Morocco	VV_Drom1765	41.05	Sudan	VV_Drom1819	24.7
Algeria	VV_Drom1373_2	78.07	Kenya	VV_Drom21	27.52	Pakistan	SRR1947236*	26.54	Sudan	VV_Drom295	22.99
Algeria	VV_Drom1379_2	79.26	Kenya	VV_Drom22	29.47	Pakistan	VV_Drom732	26.39	Syria	VV_Drom237	24.3
Algeria	VV_Drom1398_2	82.4	Kenya	VV_Drom3	29.37	Pakistan	VV_Drom735	23.45	Syria	VV_Drom280	29.76
Algeria	VV_Drom1399	39.31	Kenya	VV_Drom31	22.85	Pakistan	VV_Drom741	23.96	Syria	VV_Drom285	24.51
Algeria	VV_Drom1410	19.11	Kenya	VV_Drom32	25.71	Pakistan	VV_Drom746	20.34	Syria	VV_Drom289	23.73
Algeria	VV_Drom1416	30.72	Kenya	VV_Drom33	27.27	Pakistan	VV_Drom749	23.97	Tunisia	VV_Drom298	25.6
Algeria	VV_Drom1418	36.07	Kenya	VV_Drom34	28.46	Pakistan	VV_Drom796	26	Tunisia	VV_Drom299	21.99
Algeria	VV_Drom1422	30.13	Kenya	VV_Drom4	29.33	Pakistan	VV_Drom803	22.6	Tunisia	VV_Drom304	22.81
Algeria	VV_Drom1424	30.11	Kenya	VV_Drom5	27.53	Pakistan	VV_Drom805	26.02	Tunisia	VV_Drom309	22.34
Algeria	VV_Drom1426_2	50.08	Kenya	VV_Drom8	25.86	Pakistan	VV_Drom808	23.99	Tunisia	VV_Drom313	25.81
Algeria	VV_Drom1432	41.02	Kenya	VV_Drom9	28.79	Pakistan	VV_Drom809	25.71	Tunisia	VV_Drom315	25.61
Algeria	VV_Drom1434	30.38	Libya	VV_Drom119	21.89	Pakistan	VV_Drom947	28.82	Tunisia	VV_Drom316	25.04
Algeria	VV_Drom1435_2	74.67	Libya	VV_Drom121	26.27	Pakistan	VV_Drom963	24.96	UAE	SRR1947235*	38.59
Algeria	VV_Drom1438	26	Libya	VV_Drom139	28.16	Pakistan	VV_Drom975	22.89	UAE	VV_Drom1458	30.22
Algeria	VV_Drom1451_2	65.23	Libya	VV_Drom140	27.77	Qatar	ERR1700762*	35.29	UAE	VV_Drom1459	24.4
Algeria	VV_Drom1454	18.07	Libya	VV_Drom142	25.21	Qatar	ERR1700763*	34.87	UAE	VV_Drom1462	18.36
Austria	SRR1947234*	39.76	Libya	VV_Drom143	25.54	Qatar	ERR1700764*	37.38	UAE	VV_Drom1464	19.08
Chad	VV_Drom750	22.72	Mauritania	VV_Drom1295	26.17	Qatar	ERR1700765*	39.66	UAE	VV_Drom1468	23.03
Chad	VV_Drom752	23.12	Mauritania	VV_Drom1297	23.82	Qatar	ERR1700766*	36.35	UAE	VV_Drom1470	25.81
Chad	VV_Drom751	25.75	Mauritania	VV_Drom1302	23.43	Qatar	SRR1947230*	38.97	UAE	VV_Drom1481	22.96
Ethiopia	VV_Drom1768_2	47.55	Mauritania	VV_Drom1307	26.34	Qatar	VV_Drom357	21.59	UAE	VV_Drom1484	31.12
Ethiopia	VV_Drom1797_2	87.3	Mauritania	VV_Drom1310	31.42	Qatar	VV_Drom363	21.15	UAE	VV_Drom1485	23.64
Ethiopia	VV_Drom1800	26.62	Mauritania	VV_Drom1320	27.64	Qatar	VV_Drom367	28.17	UAE	VV_Drom1494	28.14
Iran	SRR6481367*	42.5	Mauritania	VV_Drom1335	26.88	Saudi Arabia	SRR1947231*	33.01	UAE	VV_Drom1505	25.79
Iran	SRR6481369*	41.9	Mauritania	VV_Drom1338	24.77	Saudi Arabia	SRR1947232*	38.41	UAE	VV_Drom1508	27.12
Iran	SRR6761093*	45.6	Mauritania	VV_Drom1353	24.16	Saudi Arabia	SRR1947233*	37.44	UAE	VV_Drom1511	24.2
Iran	SRR6761094*	46.4	Morocco	VV_Drom1726	39.68	Saudi Arabia	VV_Drom798A	23.6	UAE	VV_Drom1513	22.95
Iran	VV_Drom680	29.41	Morocco	VV_Drom1727	43.63	Saudi Arabia	VV_Drom799A	26	UAE	VV_Drom1526	24.19
Iran	VV_Drom692	34.32	Morocco	VV_Drom1729	36.83	Saudi Arabia	VV_Drom800A	24.08	UAE	VV_Drom1529	25.49
Iran	VV_Drom706	32.32	Morocco	VV_Drom1731	36.31	South Sudan	VV_Drom53	30.1	UAE	VV_Drom1536	28.95
Iran	VV_Drom710	31.77	Morocco	VV_Drom1733	49.98	Sudan	SRR1947238*	28.1	UAE	VV_Drom1542	28.9
Iran	VV_Drom718	33.4	Morocco	VV_Drom1737	36.8	Sudan	VV_Drom1693	24.6	UAE	VV_Drom1544	25.98
Jordan	VV_Drom373	22.06	Morocco	VV_Drom1738	48.75	Sudan	VV_Drom1696	27.8	UAE	VV_Drom1545	26.11
Jordan	VV_Drom385	29.08	Morocco	VV_Drom1741	26.01	Sudan	VV_Drom1697	23.1	USA	ERR1695771*	40.62
Jordan	VV_Drom406	23.93	Morocco	VV_Drom1742	31.32	Sudan	VV_Drom1711	26.49	USA	ERR1695772*	34.92
Jordan	VV_Drom417	25.81	Morocco	VV_Drom1746	41.27	Sudan	VV_Drom1714	23.45	USA	ERR1695822*	39.73
Kenya	SRR1947237*	26.14	Morocco	VV_Drom1748	39.71	Sudan	VV_Drom1715	24.48	USA	ERR1697181*	34.66
Kenya	VV_Drom1	24.75	Morocco	VV_Drom1749	35.63	Sudan	VV_Drom1719	25.19	Yemen	VV_Drom40	27.75
Kenya	VV_Drom10	32.33	Morocco	VV_Drom1750	46.06	Sudan	VV_Drom1720	21.4	Yemen	VV_Drom41	24.08
Kenya	VV_Drom11	27.69	Morocco	VV_Drom1754	45.45	Sudan	VV_Drom1721	23.92	Yemen	VV_Drom42	23.08
Kenya	VV_Drom15	24.92	Morocco	VV_Drom1755	49.33	Sudan	VV_Drom1722	21.95	Yemen	VV_Drom45	23.25
Kenya	VV_Drom16	28.75	Morocco	VV_Drom1757	36.89	Sudan	VV_Drom1724	26.47	Yemen	VV_Drom46	28.3
Kenya	VV_Drom18	26.8	Morocco	VV_Drom1759	43.05	Sudan	VV_Drom1725	26.19			

*Sequence data imported from public databases

Muc1 expression in both uterine horns of dromedary camel during peri-implantation window

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Introduction

In reproduction, Muc1 plays a critical role in embryo implantation and protection of mucosal epithelia from microbial and enzymatic attack (Thathiah and Carson, 2004). Therefore, in most animal species, the process of placentation is preceded by remodeling mucin-1 (Muc1) of the apical surfaces of uterine luminal epithelium (LE) (Bazer et al., 2009). In some species (mice, pig, sheep), a decrease in uterine epithelial Muc1 protein and mRNA expression accompanies embryo implantation. In other species (rabbits and humans), Muc1 appears to be locally removed at blastocyst attachment sites (Thathiah and Carson, 2004). In the alpacas, there was a reduction of the Muc1 mRNA in the left uterine horn in comparison with the right horn in the non-pregnant group at Day 15 post ovulation (PO), but no differences were seen between left and right horns of the pregnant group. However, when comparing ipsilateral horns of pregnant and non-pregnant animals, there were less expression of Muc1 in the pregnant horns in general (Barraza et al., 2018). In our previous study, applying only immunohistochemical techniques, there was spatial correlation in the expression of the progesterone receptor and Muc1 within the endometrial stroma of the dromedary camel (Al-Ramadan et al., 2013). To the authors' knowledge, no investigation has been done for the Muc1 expression in the endometrium of the dromedary camel since then. In the current work, we investigated for the first time the spatial and temporal expression of Muc1 between left and right uterine horns of non-mated and pregnant dromedary camels at days 8, 10 and 12 of gestation.

Materials and Methods

Laboratory analysis

Twelve (7-10 years) healthy females were mated with a fertile male and subdivided into 8, 10 and 12 days of pregnancy (4 animals each). Ovulation was confirmed by ultrasound and pregnancy confirmed by the presence of the embryos within the flushing fluids. Uterine biopsies were collected from both horns to perform quantitative real-time PCR (qRT-PCR), Western blot and immunohistochemistry. For the control, four she-camels were not mated and similar sampling tissues and techniques were applied.

Statistical analysis

All data was analysed by GraphPad Prism® 5 software. Means ± standard errors were calculated, and $P < 0.05$ was considered statistically significant.

Results

The results showed that Muc1 mRNA expression on Day 8 had significantly ($P<0.001$) decreased in the left uterine horn, then it increased at Day 10 before it decreased again at Day 12 compared with the non-mated camels. At the same time, there was no significant change in the level of Muc1 mRNA in the right horn at Day 8 with that of non-mated animals. Thereafter, it increased at Day 10 and peaked at Day 12 (Figure 1).

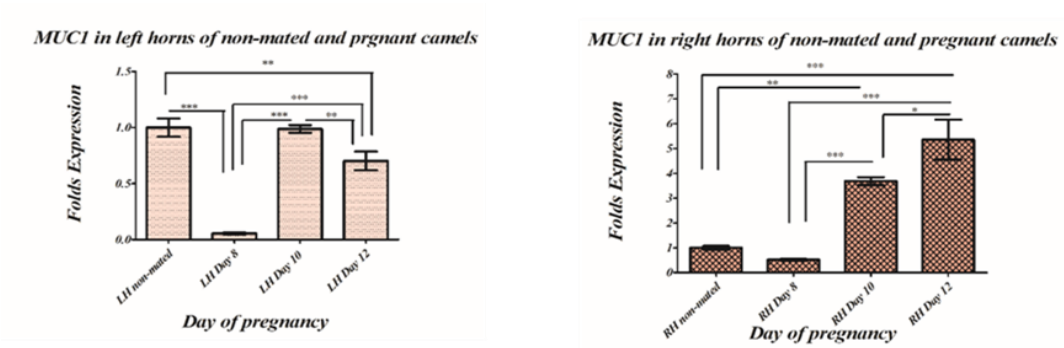

Figure 1: Muc1 mRNA expression in left and right uterine horns of non-mated and mated (Days 8, 10 and 12) pregnancy.

Analysis of mRNA within same pregnant animal groups showed that the mRNA expression was significantly higher ($P<0.001$) in the right horn compared with the left horn PO (Figure 2).

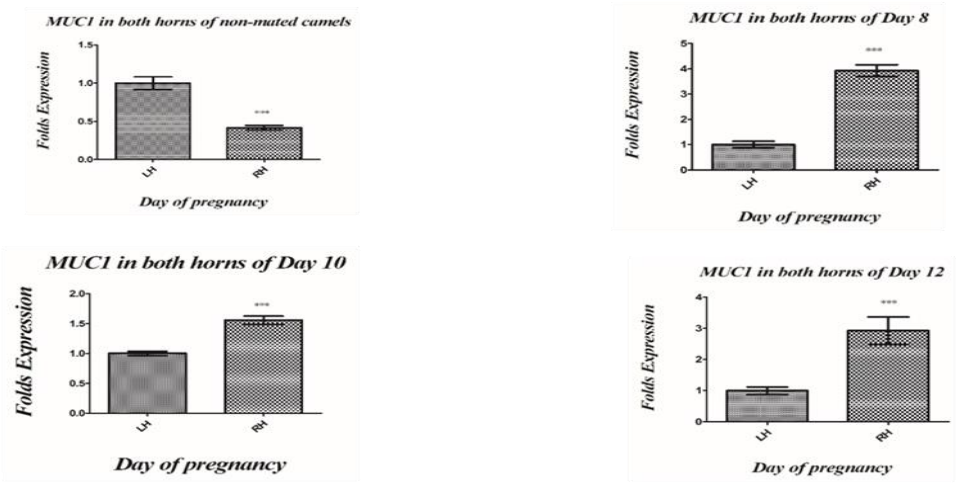

Figure 2: Comparison of mRNA expression of Muc1 between right and left uterine horns of non-mated and pregnant camels (Days 8, 10 and 12).

Western blot revealed that the 150-kDa and 180-kDa Muc1 forms were detected in all non-mated and pregnant endometrial extracts (Figure 3).

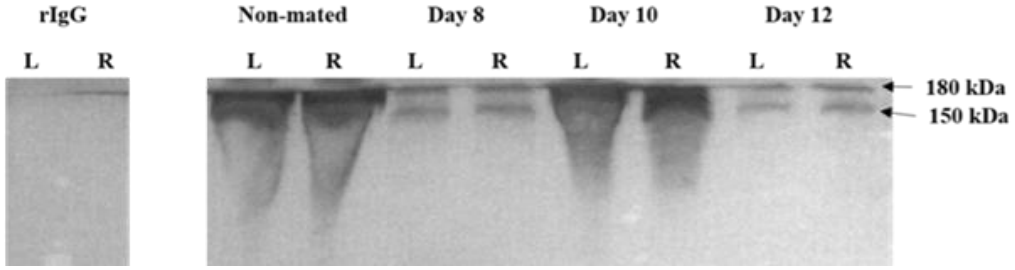

Figure 3: Western blot analysis of Muc1 of dromedary camel showed two bands of molecular masses of 180 and 150 KDa were detected.

Discussion

The current research showed that there was a sharp reduction of Muc1 mRNA level in the left uterine horn on Day 8 of pregnancy which is followed by a sharp increase at Day 10 and slight decrease on Day 12. In the right horn, however, the expression showed a gradual increase which peaks at Day 12. The embryo enters the uterus between days 6 and 7 PO and attachment starts at Day 14 PO (Skidmore et al., 1996), whilst the fetomaternal signaling is suggested to start with embryonic elongation at Day 9 PO (Tibary and Anouassi, 1997). From our data, Muc1 started to decline at Day 12 PO after reaching the peak at Day 10 PO in the left horn. This could be the beginning of further

decline over subsequent days. The images from Days 10 and 12, in the immunohistochemical study (data not displayed), showed that the luminal epithelium is devoid of any staining, or only faintly stained, whereas the glandular epithelium showed high staining intensity. Moreover, our data showed that in all days of pregnancy the left uterine horn expressed Muc1 less than the right horn. Similar data were also obtained from alpacas on Day 15 of pregnancy (Barraza et al., 2018). Therefore, the down-regulation of the Muc1 in the left horn might be a permissive for implantation. However, down-regulation of expression for Muc1 in the luminal epithelium, in most animals, is a crucial event in the early stages of pregnancy allowing for attachment and adhesion of the trophectoderm with the uterine luminal epithelium (Bazer et al., 2009). This down-regulation could be localized to the site of the embryo adhesion or where an embryonic influence triggers reduction of Muc1 expression, as is the case in rabbit and human (Carson et al., 2000). In other species, the down-regulation is generalized in the uterine epithelium and is influenced by steroid hormones (Carson et al., 2000). The data from the camel, however, suggested that the down-regulation, which is restricted to the left horn where almost all the pregnancies occur (98%), could be due to the local effect of the embryo or due to other factor(s).

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Temporospatial expression of osteopontin in both left and right uterine horns during the peri-implantation period of Dromedary camel

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Introduction

Among the major biological challenges facing camel breeders are the overall low reproductive efficiency under natural conditions, early embryonic losses, exclusive pregnancies in the left horn (98%) and pregnancy losses in the right uterine horn even in the case of twins (Skidmore, 2011). Several proteins involved in embryonic adhesion, communication and implantation processes have been studied in various mammals (Aplin, 1997). One of these impotent proteins is Osteopontin (OPN), also termed Secreted phosphoprotein 1 (SPP1). The expression of OPN in the uterine endometrium during the receptive window has been profoundly studied in several mammals (Johnson et al., 2003 and 2014). In these previous works, OPN was shown to play a vital role in the biology of reproduction. Unfortunately, no research has been conducted on the expression of OPN in the endometrium of the dromedary camel. Therefore, in the current study, we investigated the temporospatial expression of OPN and protein localization in both the left and right uterine horns of the pregnant camel during the peri-implantation period.

Material and Methods

Animals and laboratory analysis

Female dromedary camels (n=16; 7-10 years) were examined using an ultrasound scanner for detection of mature ovarian follicle (1.3-1.8 cm). Animals were then assigned to four groups (n = 4): Group-1, Group-2 and Group-3 were mated with a fertile male and samples were collected at Days 8, 10 and 12, post ovulation. Group 4 were the negative control and therefore not mated. Embryos were flushed out on the designated dates of pregnancy and biopsies were taken from right and left uterine horns from all four groups.

The biopsies were divided into two: one part was snap-frozen in liquid nitrogen and stored at -80°C for quantitative real-time PCR (qRT-PCR) and Western blot techniques, and the second part was embedded in 10% buffered formaldehyde for immunohistochemistry.

Statistical analysis

All data was analysis by GraphPad Prism®5 software. Means ± standard errors were calculated, and $P < 0.05$ was considered statistically significant.

Results

Expression of OPN mRNA

Results of qPCR confirmed presence of OPN mRNAs in the endometrium of both non-mated and pregnant camels. The endometrial expression of OPN was significantly higher ($P<0.001$) in both horns on Days 10 and 12 of pregnancy compared with that of the non-mated females (Figure 1). However, there was no difference in OPN expression between Day 8 of pregnancy and the non-mated camels. Compared to the non-mated females, OPN expression on Day 10 increased 14.5-fold in the left uterine horn, but only 8.4-fold in the right uterine horn ($P<0.001$). At Day 12 of pregnancy, the expression of OPN continued its sharp increase to 37.4-fold in the left uterine horn, whereas it had decreased to 7-fold in the right uterine horn compared with non-mated females ($P<0.001$). Meanwhile, OPN expression was significantly less ($P<0.001$) by about two thirds of that in the left uterine horn. However, no significant differences in OPN mRNA levels were detected between the left and right uterine horns at Day 8 and Day 10 of pregnancy, or in the non-mated females. In comparison, OPN expression had significantly ($P<0.001$) increased 13.8-fold at Day 10 and 36.7-fold at Day 12 in the left uterine horn, but only increased 7.9-fold at Day 10 and 6.5-fold at Day 12 in the right uterine horn, compared with Day 8 of pregnancy. Moreover, OPN expression was significantly ($P<0.001$) increased 22.9-fold at Day 12 in the left uterine horn, whereas, there was no significant difference in the right uterine horn at Day 12 compared with Day 10 of pregnancy.

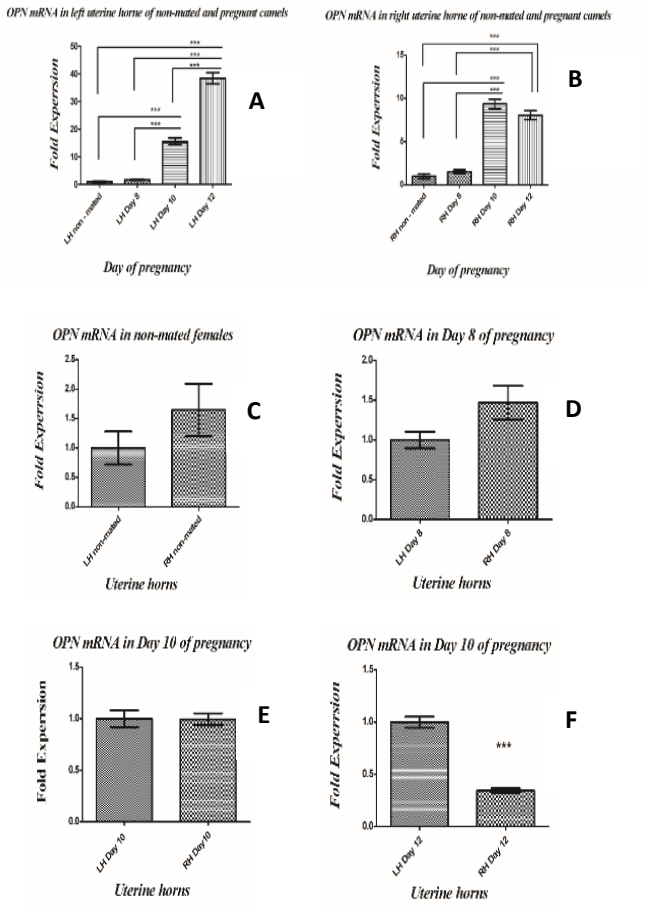

Figure 1: OPN mRNA expression in non-mated and pregnant camels. **A:** OPN mRNA expression in the left uterine horn of non-mated and pregnant camels. **B:** OPN mRNA expression in the right uterine horn of non-mated and pregnant camels. **C:** Differences in OPN mRNA expression between left and right uterine horn in non-mated females. **D:** Differences in OPN mRNA expression between left and right uterine horn on Day 8 of pregnancy. **E:** Differences in OPN mRNA expression between left and right uterine horn on Day 10 of pregnancy. **F:** Differences in OPN mRNA expression between left and right uterine horn on Day 12 of pregnancy.

Detection of OPN protein

The Western blot detection of OPN protein in the endometrium of non-mated and pregnant camels is shown in Figure 2. Only the 70-kDa OPN form was detected in all non-mated and pregnant endometria, with increased intensity noted in pregnant camels compared with non-pregnant camels. Notably, the immunointensity of OPN protein was relatively low on Day 8, increased at Day 10, and peaked at Day 12 of pregnancy compared with that of the non-mated camels. At the same time, the left uterine horn showed higher intensity of OPN protein compared with the right horn in all non-mated and pregnant camels.

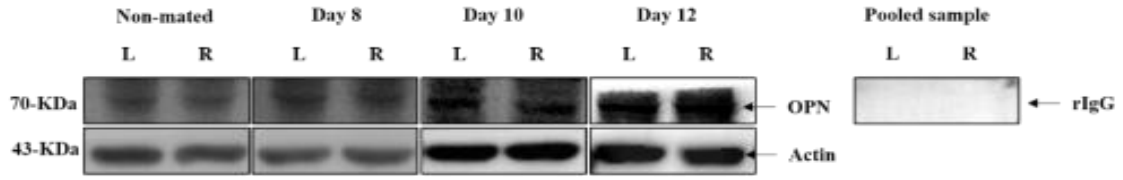

Figure 2: OPN protein detection by western blot in both left (L) and right (R) uterine horns in the non-mated and pregnant camels.

Discussion

The present study determined for the first time the spatial and temporal expression of OPN in pregnant dromedary camels during the peri-implantation window. The results showed that OPN mRNA and OPN protein were constitutively expressed in non-mated and pregnant camels. However, OPN expression was upregulated as pregnancy advanced. The current detection of OPN in the uterus has also been demonstrated in other mammalian species even though they have different placentation and implantation processes (Berneau et al., 2019; Johnson et al., 2003). We observed a similar pattern of increasing OPN expression as pregnancy advanced in dromedary camels but with different dates of onset. These differences may be explained by innate biological differences between mammals, but it is a mystery why implantation in camelids occurs exclusively in the left uterine horn (98%) (Tibary and Anouassi, 1997).

In the present study, only the 70-kDa OPN form was detected in all non-mated and pregnant endometria, with apparent gradual increase from non-mated to advanced pregnancy stages, and with the left uterine horn showing increased intensity compared with the right uterine horn. In pigs, both 70- and 45-kDa OPN forms have been detected in all cyclic and pregnant endometria and was upregulated with increasing gestational age (Garlow et al., 2002). Similarly, in sheep endometrial extracts all OPN forms (70, 45, and 24-kDa) were detected, and did not differ, between cyclic and pregnant animals (Johnson et al., 1999).

The currently identified OPN protein pattern was consistent with OPN mRNA expression; higher OPN protein immunoreactivity was detected in the left compared with the right horn in all pregnant camels and increased with gestational age. In the dromedary camel, the migration of the free, elongated embryo toward the left uterine horn occurs between Day 8 and 10 of post-ovulation and the attachment of trophoblast occurs at Day 14 post ovulation (Tibary and Anouassi, 1997). The gradual increase in OPN concentration in the left uterine horn from non-mated to Day 12 of pregnancy may play a role in attracting the embryo to migrate into the left horn and release its maternal recognition of pregnancy signal to prevent luteolysis or trigger a luteotrophic process (Skidmore, 1996).

In conclusion, OPN mRNA and protein were detected and upregulated in dromedary camels during the peri-implantation period. In addition, OPN was more highly expressed in the left uterine horn than in the right at the time of implantation and attachment (Day 12).

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Genetic contribution of myogenicfactor 5 and growth hormone genes for live body measurements, carcass traits and meat quality of dromedary camel

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Improvement of camel meat quality would likely increase consumer acceptance and perception of meat. Although great progress has been made in animal breeding in the last few decades, meat quality traits have had less attention due to the difficulty of obtaining the phenotypes, which in most of cases are measured after slaughtering (Andersson, 2001). Moreover, Miar et al. (2014) noted that meat quality traits have low-to-moderate heritability, while carcass composition traits have moderate-to-high heritability. Therefore, with the wider applications of molecular marker technology, the improvement of meat quality became accessible (Gao et al., 2007).

The objectives of the present study were to define the variations in the region of exon 1 of MYF 5 and two regions of GH 3 UTR and GH 5 UTR genes in dromedary camels. An additional objective was to study the association between the detected single nucleotide polymorphisms (SNPs) in these regions and live body measurements, carcass traits, carcass cuts, histological traits and chemical composition of the *Longissimus dorsi* muscle.

In the current study association analyses between identified SNPs in myogenic factor 5 (MYF5) and growth hormone (GH) genes and live body measurements, carcass traits and meat quality of dromedary camel were performed. A region of exon 1 of MYF5 and two regions from GH (GH 3 UTR and GH 5 UTR) genes were screened to detect the SNPs in dromedary camels. The results indicated that identified SNPs in MYF5 and GH had significant effect on numerous body measurements, carcass traits, carcass cuts, histological traits and chemical composition. The three regions were found to be associated with several meat characteristics and it is recommended they be taken as candidate genes for meat characteristics in dromedary camels. Further studies with a

larger sample size are required to confirm the effects of MYF5 and GH genes on growth traits and meat quality in camels.

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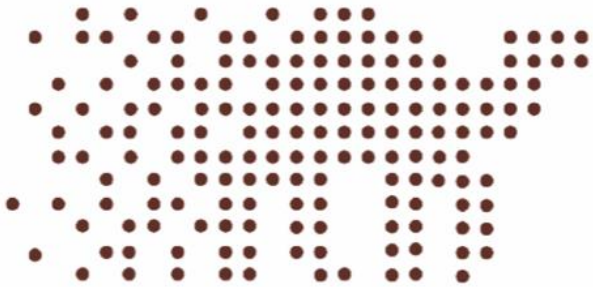

- ICO vision:
  - Preservation, restoration, revival, development & promotion of the culture of camel breeding globally
- ICO mission:
  - To contribute to preservation of ethno-cultural heritage through development of camel breeding culture
- Global promotion of camel principles of practice & activities:
  - Encouragement of scientific research
  - Principle of humane attitude towards camels
  - Cooperation with international organizations
  - Fair competition & doping controls
  - Organisation of ICO World Festivals

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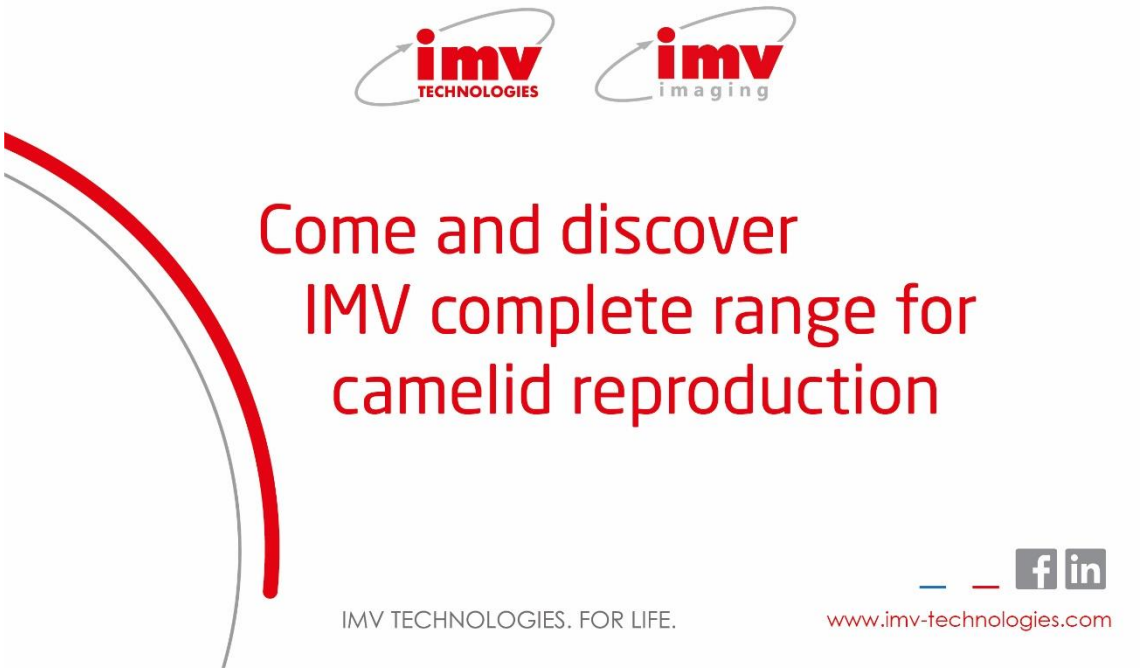
أهداف ووظائف المنظمة

Goals and functions of the ICO

- Promotion of mutual understanding and friendship between nations and people, establishment of societies of nature and animal lovers through various practices with camels.
- Development of the WCM in all its forms and types and everything related to interaction of humanity and camel.
- Organize and host the ICO WF.
- Development and management of the ICO code of conduct as a set of moral principles, knowledge, technical and basic skills of the camel culture.
- Promotion of research aimed at scientific and educational development of the camel culture and provide consultation and advice on all matters relating to camel affairs.
- Promoting and encouraging the establishment of national and regional member organizations of the ICO. Organization of training workshops for members of these organizations.
- Integration of friendly organizations and institutions of national, regional, continental and international measures in the ICO, which lead their activities in the field of camel breeding.
- Achieving the excellence by all ICO members, organizers, participants, involved in camel activities in all areas, such as technical equipment, cultural, social, sports events, development of talent in the field of camel breeding and their activities, industry and science.
- Maintenance of international rules and principles of the camel breeding practice, establishment of necessary Provisions in accordance with this Statute of the ICO, instructions and principles established by the relevant international organizations.
- Spreading and expanding the practice base, equipment and location of camel breeding sites and farms, optimizing methods of implementation and search for suitable locations in coordination with the competent authorities in each country.
- Issuing of certificates for registration of camels.
- Restoration of historical and creation of new caravan competitions, creation of new caravan routes, travels and excursions.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German Standard Group (GSG) is a frontrunner in the UAE veterinary industry with a growing range of veterinary equipment. We market veterinary equipment and instruments exclusively for veterinary practices across the region and strive to offer the most up-to-date veterinary supplies to the veterinary community, enabling them to cater to the health concerns of their customers better, and therefore conduct a more cost-effective and profitable business.

VISION

To be the leader and most respected distributor for veterinary products and animal health care services that exceed customer expectations and satisfaction. To contribute on education and enhancement of the quality, preservation, and welfare of animal life. We are also working hard to provide a wider platform for the training and further education of professionals in the animal care and veterinary industries across the GCC and beyond.

MISSION

To foster continuous education, training and people empowerment for the customers and the GSG workforce. To provide exceptional customer service through integrated effort, commitment, dedication and integrity. To continuously endeavor to deliver quality products for animal care and welfare from different markets across the world. To apply our coherent intelligence, initiative, experience and skills to the expansion of veterinary animal health care.

GOALS

Be fully committed to the client, create a trustworthy platform between the client and the company. Training and Education is a major goal so German Standard Group is focusing on both indoor and outdoor training. German Standard Group is working hard on offering all the veterinary supplies from consumables & disposables to large machinery equipment. German Standard Group is also an up-to-date company that understands what the market needs and fills the present gap.