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EFFECT OF BUSERELIN ON FOLLICULAR DYNAMICS AND HORMONAL SECRETORY PROFILE IN VICUNA (*VICUGNA VICUGNA)*

A. Agüero[1], M.A. Aba[2], E.F. Capdevielle[1], M.G. Chaves[1], B. Rutter[1], C. Bianchi[2] and M.H. Miragaya[1]

[1] Área de Teriogenología, Facultad de Ciencias Veterinarias,Universidad de Buenos Aires. Av. Chorroarín 280, 1427 Capital Federal, Argentina [2] Área de Endocrinología, Facultad de Ciencias Veterinarias, Universidad del Centro de la Provincia de Buenos Aires, Campus Universitario, Paraje Arroyo Seco, Tandil-7000-Buenos Aires, Argentina

ABSTRACT

The aim of the study was to evaluate the effect of an injection of the GnRH analogue buserelin in vicunas with a dominant follicle. Follicular dynamics was monitored daily during 12 days by transrectal ultrasonography and blood samples were collected on each day in non-pregnant, non lactating vicunas. In treated animals, the day a dominant follicle was first detected, 8 mg of buserelin was injected intravenously. Mean follicle diameter the day of buserelin injection (day 0) was 7.9 ± 0.3 mm. Thereafter, mean follicle diameter decreased to 5.4 ± 0.9 mm, on day 1, 3.2 ± 0.35 mm on day 2, and varied between 3.5 and 2.2 mm until day 7 when the lowest mean follicle size (2.2 ± 0.21 mm) was recorded. In control animals, after attainment of dominance (day 0) follicles remained growing until day 1 and thereafter started to regress. In addition, all control animals showed development of a new follicular wave immediately after the regression of the initial one. Conversely, no dominant follicles were recorded in treated animals after ovulation. Mean progesterone concentrations remained low (around 1 nmol l^{-1}) until day 4 after treatment, when they started to sharply increase. Peak concentrations (30.2 ± 9.5 nmol l^{-1}) were recorded on day 8 after buserelin injection and had decreased to 8.1 ± 2.5 nmol l^{-1} by day 9. In control animals, progesterone concentrations remained close to 1 nmol l^{-1} throughout the study. In treated animals, maximum oestradiol-17β plasma concentrations were recorded on day 0 (38.3 ± 4.2 pmol l^{-1}). Thereafter, mean plasma concentrations steadily decreased until day 3 when its lowest concentrations were recorded (18.2 ± 1.8 pmol l^{-1}). On day 4, mean concentrations started to increase until day 6 (28.0 ± 3.5 pmol l^{-1}). In control animals, concentrations remained close to those recorded on day 0 until the end of the study.

In conclusion, buserelin injection in the presence of a dominant follicle induces ovulation in vicunas. In addition, the presence of endogenous progesterone from an active corpus luteum was shown to have a negative effect on follicle dynamics. This action could be used in future to synchronise the emergence of a new follicular wave.

Key words: Buserelin, follicular dynamics, oestradiol-17β, progesterone, vicuna

South American camelids are induced ovulators, the process of ovulation being naturally triggered by copulation. The injection of exogenous hormones (hCG, GnRH) have proven to be effective to induce ovulation. San Martín *et al* (1968) reported that, in the alpaca, copulation and hCG administration produced ovulation 24 and 26 hours afterwards respectively. Fernández Baca *et al* (1970) evaluated different stimulus and observed that copulation using either a whole or vasectomised male produced high percentages of ovulation.

Administration of hormones to induce ovulation has also been studied. Thus, it has been shown that llamas ovulate after administration of 500 to 1,000 IU of hCG, either IV or IM (Johnson, 1989). Meanwhile, gonadotrophin releasing factor, gonadorelin, induced ovulation at a dose of 1,000 mg (Bravo *et al*, 1992) and the GnRH analogue buserelin at a dose of 4-8 mg produce the same effect (Sumar *et al*, 1981). Buserelin has been used to synchronise the emergence of new follicular waves in llamas as a treatment prior to ovarian superstimulation and embryo recovery (Ferrer *et al*, 2002).

Urquieta *et al* (1995) reported the possibility of inducing ovulation in vicunas by injecting a GnRH analogue. They observed that after GnRH treatment, progesterone plasma concentrations increased in some animals from day 4, reaching a maximum of 4.4 ± 0.8 nmol l^{-1} between days 5 and 8 while in other animals no changes were recorded, but to our knowledge, no data exists on endocrine changes related to ovarian dynamics evaluated using ultrasonography.

SEND REPRINT REQUEST TO A. AGÜERO email: aguero@fvet.uba.ar

The objective of this study was to evaluate the effect of injecting a GnRH analogue in the presence of a dominant follicle by monitoring the variations in plasma concentrations of progesterone and oestradiol-17β and its influence on follicular dynamics in vicunas.

Materials and Methods

Animals

Seventeen adult, non-pregnant and non-lactating female vicunas were captured and kept isolated from males during the study. The animals were fed bales of alfalfa and water *ad libitum*. The study was carried out in February and March, at the Experimental Station Reserva de la Biosfera, Laguna Blanca, in the province of Catamarca, Argentina. This station is located 26º 57′ South Latitude, 66º 33′ West Longitude at 3,165 m above sea level.

Experimental procedure

Follicular dynamics was monitored daily during 12 days, by transrectal ultrasonography, using a real-time B mode scanner (LC 2010 plus Berger, Buenos Aires, Argentina) with a 5 MHz linear-array electronic transducer and provided with a 30 cm handle due to the impossibility to introduce the hand of the operator into the rectum. The faeces were removed and the ultrasound gel was previously introduced into the rectum to enable scanning directly in the rectum. In 13 animals, the day a dominant follicle (larger than 6 mm, according to the definition of Miragaya *et al*, 2004) was first detected, 8 mg of buserelin (Receptal®, Intervet Argentina) were injected intravenously. Considering dominance was attained on different days, the period from buserelin injection to the end of the study varied from 5 to 9 days in different animals. Thus the number of treated animals decreased from 12 on day 5 to 3 on day 9. Four animals remained as controls.

Blood samples were collected daily by jugular venipuncture into heparinised tubes (Vacutainer®, Becton Dickinson and Company, Franklin Lakes NJ, USA). Plasma was immediately separated by centrifugation at 2,000 g and stored at –196ºC in a liquid nitrogen container until analysis.

Hormone assays

Progesterone concentrations were determined by an enzyme immunoassay previously validated for use in llama plasma (Aba *et al*, 2001). Serial dilutions of vicuna plasma containing high concentrations of progesterone produced curves parallel to the standard. The intra-assay coefficient of variation was around 13% at 1.2 nmol l^{-1} and remained below 12% for concentrations up to 159 nmol l^{-1}. The inter-assay coefficient of variation for control samples containing 5 nmol l^{-1} and 80 nmol l^{-1} was 7 and 11%, respectively. The sensitivity of the assay defined as the intercept of maximal binding –2SD, was 0.8 nmol l^{-1}.

Oestradiol-17β was determined using an RIA previously validated for use in bovine plasma (Sirois and Fortune, 1990) with the following modification: the standard curve was prepared with standards supplied with the radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Serial dilutions of vicuna plasma with high concentrations of oestradiol-17β produced curves parallel to the standard. The intra-assay coefficients of variation, calculated from the precision profiles, were 12.8% at 6 pmol l^{-1}, 12.2% at 17 pmol l^{-1} and below 11% up to 180 pmol l^{-1}. The inter-assay coefficients of variation for 3 control samples were 21% (13 pmol l^{-1}), 9% (39 pmol l^{-1}) and 12% (84 pmol l^{-1}). The lowest amount of estradiol-17β detectable (defined as the intercept of maximal binding-2 SD) was 4 pmol l^{-1}.

Analysis of data

Data were analysed using a one way Kruskal-Wallis test in order to evaluate differences in the maximum follicular size and plasma concentrations of oestradiol-17β and progesterone. Results are expressed as mean ± SEM. Differences were considered significant at $p<0.05$. Data corresponding to hormone concentrations and follicular size were normalised to day of detection of a dominant follicle for further processing of the results.

Results

Follicular dynamics

All animals showed ovarian activity at beginning of the study. Mean follicle diameter the day buserelin was injected (day 0) was 7.9 ± 0.3 mm ($p=0.15$). On day 1, ovulation occurred in 7/12 vicunas and on day 2, all treated females were detected ovulated. Thereafter, mean follicle diameter decreased to 5.4 ± 0.9 mm on day 1 ($p=0.39$), 3.2 ± 0.3 mm on day 2 ($p=0.01$) and varied between 3.5 and 2.2 mm until day 7 ($p=0.02$) when the lowest mean follicle size was recorded, i.e. 2.2 ± 0.2 mm. In control animals, after attainment of dominance (day 0) follicles remained growing until day 1 and thereafter started to regress. In addition, all control

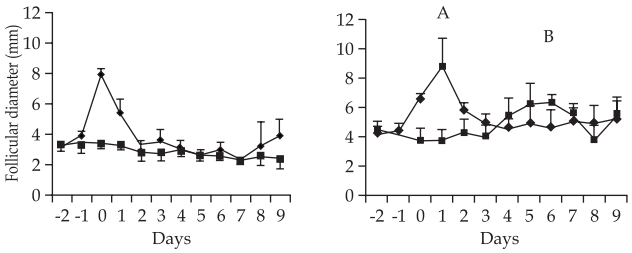

Fig 1. Follicular dynamics in treatment group (ovary with dominant follicle at the moment of buserelin injection: closed diamond, and contralateral ovary: closed square) and in control group (ovary with dominant follicle from wave A normalised at day of attainment of dominance (day 0): open diamond, and contralateral ovary with wave B: open square).

animals showed development of a new dominant follicle (wave B) after the regression of the initial one (wave A). Conversely, no dominant follicles were recorded in treated animals after ovulation induced by buserelin injection (Fig 1). One animal did not respond to buserelin treatment and developed a hemorrhagic/cystic follicle that reached a diameter of 25.4 mm and persisted throughout the experiment. Data from this animal was excluded from the analysis.

Oestrogen secretory profile

In treated animals, peak oestradiol-17β plasma concentrations were recorded on day 0 (38.3 ± 4.2 p mol l^{-1}) (p=0.80). Thereafter, mean plasma concentration steadily decreased until day 3 when its lowest concentration was recorded (18.2 ± 1.8 pmol l^{-1}) (p=0.003). On day 4, mean concentration started to increase until day 6 (28.0 ± 3.5 pmol l^{-1}) (p=0.12). In control animals, concentrations remained close to those recorded on day 0 (34.4 ± 3.3 pmol l^{-1}) until the end of the study (Fig 2).

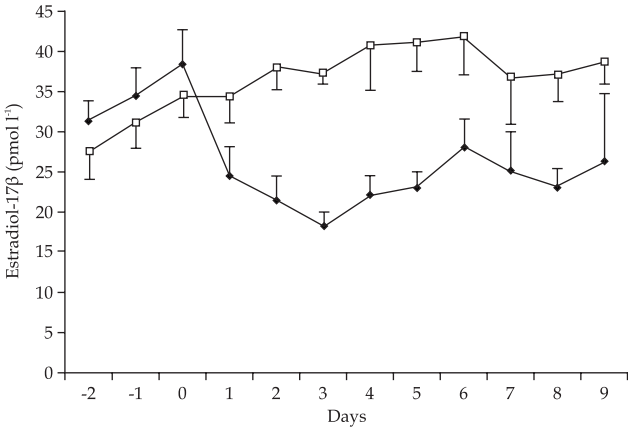

Fig 2. Oestradiol-17β levels in treatment group (closed diamond) and control group (open square).

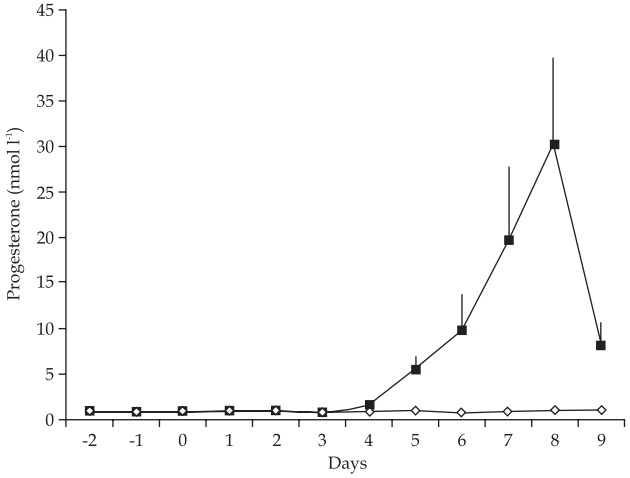

Fig 3. Progesterone levels in treatment group (closed square) and control group (open diamond).

Progesterone secretory profile

The presence of a functional corpus luteum after induction of ovulation was retrospectively confirmed by the increase in progesterone plasma concentrations in 12 out 13 animals after treatment. Conversely, attempts to identify ultrasonographically the presence of luteal structures were unsuccessful.

Mean progesterone concentrations remained low (around 1 nmol l^{-1}) until day 4 after treatment, when they started to sharply increase. Peak concentrations were recorded on day 8 after buserelin injection (30.2 ± 9.5 nmol l^{-1}) (p=0.02) and had decreased to 8.1 ± 2.5 nmol l^{-1} by day 9. In control animals, progesterone concentrations remained close to 1 nmol l^{-1} throughout the study (Fig 3).

Discussion

Buserelin injection resulted in ovulation in 12/13 animals. Ovulation was confirmed by the rapid reduction in follicle size, the drop in oestradiol-17β plasma concentrations registered one day after injection and the increase in progesterone concentrations observed after day 4.

The secretory profile of progesterone after buserelin injection is similar to that reported in other camelids after induction of ovulation (Aba *et al*, 1999). Despite the low number of animals, from the present results, a corpus luteum life span of 8 days might be suggested in vicunas, similar to that reported for the species by Urquieta *et al* (1995) and 1 to 2 days shorter than reported in other South American camelids (Sumar *et al*, 1988; Adams *et al*, 1990). In addition, plasma progesterone concentrations recorded in the present study are

higher than those reported previously (Urquieta *et al*, 1995). Differences might be attributable to the use of different methods for hormone determination between studies.

The pattern of follicular growth and regression recorded in control animals resembled that previously reported in vicunas (Miragaya *et al*, 2004). The disclosure that the mean follicle size in wave B was smaller than that recorded in wave A, might be related to the fact that peak follicle size was attained on different days, while the first wave was normalised at day of attainment of dominance (day 0).

The observation that no follicle developed to dominance after ovulation in treated animals suggests that progesterone from the corpus luteum exerts a negative influence on follicular growth. This hypothesis is further supported by the low oestradiol-17β plasma concentrations recorded after buserelin injection. This is in agreement with previous reports indicating a similar negative influence of exogenous progesterone on follicular activity in vicunas (Aba *et al*, 2005) and llamas (Aba *et al*, 1999; Chaves *et al*, 2002). Conversely, follicles reached ovulatory size during wave B while oestradiol-17β plasma concentrations remained high during the same period in control animals due to the tendency of regressing and growing follicles to overlap in vicunas (Miragaya *et al*, 2004).

Ferrer *et al* (2002) showed that the ovulatory effect of buserelin in llamas could be used to synchronise the emergence of a new follicular wave in all treated females. This observation could be compatible with the results hereby reported, suggesting that the application of buserelin in vicuna could be an interesting tool used to synchronise a new follicular wave.

In conclusion, buserelin injection in the presence of a dominant follicle induces ovulation in vicunas. The lifespan of the newly formed corpus luteum might be estimated in 8 days. Moreover, the presence of endogenous progesterone from an active corpus luteum was shown to have a negative effect on follicle dynamics. This action could be used to synchronise the emergence of a new follicular wave.

Acknowledgements

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Bovine virus diarrhoea in camels: role of camels infected with bovine viral diarrhoea virus in transmission of the disease

The study was conducted to investigate bovine diarrhoea virus (BVD) in camels and to determine the role played by camels in the transmission of BVDV to cattle. 50 cattle in contact with 50 camels were subjected to clinical, serological, virological and biotechnological examinations. Initial laboratory investigations confirmed that all the cattle were free from BVD at the beginning of the study. Clinical examination, indirect ELISA, virus isolation and reverse transcription-polymerase chain reaction (RT-PCR) were performed for each animal. Indirect ELISA, virus isolation and RT-PCR were carried our twice, one month apart. No clinical signs were observed in camels, although some animals were positive for the presence of BVDV in one or more of the previosly mentioned tests (except 2 camels that showed severe signs). In cattle, 14 out of 23 infected animals suffered from clinical signs 3 weeks after the study was started, whereas the rest of the animals were apparently healthy. All examined camels were negative in the 1st and 2nd ELISA except for 2 camels that showed a weak positive result in the 2nd ELISA. No cattle were positive in the 1st ELISA, but 16 cattle were positive in the 2nd ELISA. BVDV was obvserved from 11 camels in the 1st and 2nd isolation, whereas all examined cattle werer negative for 1st virus isolation. However, BVDV was isolated from 17 cattle in the 2nd virus isolation. In the first RT-PCR, BVD viral nucleic acid (RNA) was detected in 15 camels. No BVD viral nucleic acid awas detected in the all cattle examined during the 1st RT-PCR, whereas in the 2nd RT-PCR viral necleic acid of BVD was detected in 15 camels and 23 cattle. Camels and cattle that gave a positive result in ELISA and virus isolation were positive with RT-PCR. Results of this study proved that camel could get infected with BVDV without showing clinical signs. Thus, it could transnmit the virus to cattle through contact even for a relatively short time and remain infective for a long time without observation. RT-PCR technique seemed to be more sensitive than ELISA and virus isolation in the diagnosis of persistent form of BVD, whereas virus isolation was more sensitive than ELISA. Camels could play a very important role in the persistence and transmission of BVDV infection among cattle. Therefore, any epidemiological studies on BVDV and control programme planning should put this point of view in consideration. At the same time, RT-PCR techniques seemed to be very sensitive and suitable for the diagnosis of BVDV infection in cattle and camels, especially when other tests failed to detect the infection. Therefore, this technique is recommended for use in the screening of camels (especially those improrted from Sudan) for their freedom from BVDV. This is the first study on the role of camel in the transmission of BVDV to catttle and the first to use RT-PCR assay in the diagnosis of BVDV in camels, in addition to the first recognition of BVDV genotype II in Egypt.

(El-Hakim UA, Assiut Veterinary Medical Journal (2004)50(102)106-121)
Courtesy: CAB International, UK

NEWS

Robot Jockey

A camel ridden by a remote controlled robot jockey of Swiss Robotik Platforms for Research and Education company K-team agt a test race in Chalet-a-Gobet in Switzerland on 10th September 2005. The news was published in a national daily (see the cover page photo). The Qatar Government has approached K-team to deliver 150 robot Jockeys for the popular camel races.

Source: The Hindu, 12 September 2005.

Adventure Safari with Bactrian Camels

Four bactrian camels of Kabul breed were purchased with the help of Laddakh tourism at Kashmir by a team of five tourists belonging to Australia, France and United Kingdom to travel from Rajgarh to Pushkar of Rajasthan state, India.These camels were transported from Laddakh to Rajgarh on trucks and after a brief rest they started their journey on foot. Two camels fell sick at Bikaner and were treated by a team of doctors at College of Veterinary and Animal Sciences, Bikaner but could not save one camel which died. Other camel was also critically ill but was removed from the hospital by these tourists. Local people hired to escort these camels told that other sick camel also died few days later. District Administration ordered an enquiry to probe the reasons of death following a complaint lodged by animal activists. District administration advised these tourists to transport the surviving camels back to their natural habitat.

Source: Dr. T.K. Gahlot, Editor, JCPR and Dainik Bhasker 21 September 2005)

New Children's Book Empowers Children with Cancer and Raises Awareness for the Endangered Wild Bactrian Camel

"Bradford and the Journey to the Desert of Lop" has been awarded Dr. Toy's 100 Best Children's Products for 2005.

ISBN: 0-9761768-2-3
Written by Dawn Van Zant
Illustrated by Alexander Levitas

The new line of children's non violent toys and books produced by Wild Heart Ranch empowers children with cancer and brings awareness to the endangered wild Bactrian camel. The wild camels living in the far away Gobi Desert may offer a possible cure for illness based on preliminary research. The book teaches us that, "One small boy with cancer can change the world if you listen."

Follow Brad and the Sandman on their magical journey to the Desert of Lop to learn the secrets of the desert that live in your heart.

The Wild Camel Protection Foundation, a United Kingdom registered charity, with Jane Goodall as its patron, was established in 1997. It is also registered in the USA as a non-profit organisation. The sole aim of the Wild Camel Protection Foundation is to protect the critically endangered wild Bactrian camel (Camelus bactrianus ferus) and its habitat in the fragile and unique desert ecosystems in the Gobi and Gashun Gobi deserts in Northwest China and South West Mongolia. Supporting awareness for the Wild Camel Protection Foundation at www.wildcamels.com