Fate of the unfertilized oocytes in alpacas

Yessenia Picha,[a] Julio Sumar,[b] Mushtaq A. Memon[a]
[a]Department of Veterinary Clinical Sciences, Washington State University, Pullman, WA; [b]IVITA Research Station, La Raya, San Marcos University, Cusco, Peru

Abstract
Fourteen mature female alpacas between three and six years of age were assigned to two experiments that were designed to determine the fate of unfertilized alpaca ova. In experiment one, 12 alpacas were given 50 ug of gonadotropin releasing hormone (GnRH) to induce ovulation. Ovulation was detected by transrectal ultrasonography (TUS). Alpacas were sacrificed, the oviducts and uterine horns were flushed and ova were identified under a stereo microscope. In experiment two, two alpacas having no dominant follicle, or corpus luteum, were given 400 IU of equine chorionic gonadotropin (eCG), monitored by TUS, and when the follicles reached 6 mm diameter, 75 ug of GnRH was given to induce ovulation. Animals were sacrificed seven days after ovulation induction and uterine horns were flushed. Results from experiment one show that at one day after ovulation, oocytes in the oviduct were of normal morphology and surrounded by cumulus oophorus cells. At two days after ovulation, the oocyte showed a clear, shrunken, dissociated cytoplasm as well as unequal cell division. At day three after ovulation, ova were surrounded by cumulus oophorus cells similar to those observed on day one after ovulation, as well a completely disorganized and clear cytoplasm. By day six after ovulation, ova were shrunken with broken zona pellucida. Results of experiment two show that all ova collected from the uterus were degenerated, but still surrounded by cumulus oophorus cells. In conclusion, unfertilized ova are not retained in the oviduct of the alpaca.

Keywords: Alpaca, unfertilized ova; oocyte collection; ovulation induction.

Introduction

Female South American camelids (SAC) present striking reproductive peculiarities compared to other domestic livestock: induced ovulation, short half-life of the corpus luteum (CL), and different luteolytic activity between the two uterine horns resulting in establishment of exclusively left horn pregnancies.[1-3] Scientific literature describing the fate of the unfertilized mammalian ova is scarce. Degeneration and disappearance of the ova in the oviduct is considered a possibility. Knowledge of the fate of unfertilized ova, including its morphological description, may shed some light on physiological processes. Van Nierke and Gerneke[4] first drew attention to the differential transport of oocytes and embryos in the equine oviduct. Namely, if the freshly ovulated oocyte remains unfertilized, it passes down the oviduct only to the ampullary-isthmus junction where it remains lodged in the highly convoluted folds of oviductal mucosa and degenerate slowly over many months. Bouker et al[5] suggested that in llamas, the non-fertilized oocyte does not reach the uterus after ovulation implying that camelids are similar to horses with respect to selective embryo transport through the oviduct. In mares, embryo transport through the uterine tube and especially through the utero-tubal junction (UTJ) into the uterus seems to be under the influence of the embryo.[6] The mechanisms controlling embryonic descent into the uterus are not understood in alpacas. Our hypothesis was that unfertilized or defective oocytes are retained in the oviduct, and embryo hatching from the zona pellucida needs to occur within the UTJ in order to permit passage of the embryo. The main objective of the study was to determine the fate of unfertilized oocyte in alpacas.

Material and methods

Animal selection

A total of fourteen female alpacas destined for meat production were selected for the study in experiment one (n=12) and for experiment two (n=2). All animals were between 3 and 6 years age (median = 5 years) and had a cria the previous year. The study was conducted during August (dry season

in Peru). Animal selection criteria for inclusion in the study were good general health, no prior reproductive disorders, and a body condition score between 3 and 3.5 (1-very thin, 5-obese). All females were maintained at La Raya Research Station, Cusco, Peru, and were maintained under natural pasture, supplemented with a mix of oat hay and dry corn straw (500 g per animal) for maintenance and had free access to fresh water.

Ovulation induction and ovarian superstimulation management

All females were examined by transrectal palpation by one veterinarian with small hands to determine if there were any gross abnormalities,[7] and to verify the uterine tone and edema.

In experiment one, follicular activity was monitored daily by TUS using 7.5 MHz linear transducer. When a dominant single follicle was between 7 and 10 mm in diameter, and the uterus showed maximum tone and edema, the females were treated with 50μg of GnRH for induction of ovulation. To determine the time of ovulation, females were examined by TUS every four hours starting 24 hours after the administration of GnRH. After confirmation of ovulation (disappearance of the follicle), females were randomly assigned to a group for oocyte collection at day 1, 2, 3, 4, 5 and 6 post-ovulation (day 0 = ovulation). The numbers of animals for each collection day were two.

The two females of experiment two were treated with a superovulation protocol. When the alpacas did not have any dominant follicle (smaller than 3 mm) and no CL present, were given a single dose of 400 UI of eCG The alpacas were monitored by TUS to evaluate follicular development on day 2, 4 and 6 after eCG administration. When follicles reached 6 mm diameter, alpacas were given 75 ug of GnRH to induce multiple ovulations. Animal were sacrificed seven days after ovulation.

Ovulation and corpus luteum formation

In experiment one, eight females ovulated, and the CL was not detected before day 4 after ovulation. In our experience the CL can be imaged properly at four days after ovulation (five days after mating). In experiment two, there were three (10, 11, and 11 mm), and four corpora lutea (9, 10, 10 and 12 mm), in each of the females. In both cases the CLs were imaged properly.

Postmortem reproductive tract collections and examination

All females were slaughtered according to the approved methods set forth by the Peruvian veterinary authorities. Following slaughter, the entire reproductive tracts were removed *en block* and immediately transported to the laboratory. Since the slaughter facility was located in the same building that the laboratory, transportation of the reproductive tracts, took approximately two minutes.

The number of CLs and the side of ovulation (left and right) were correlated with the number and side of the follicles observed in each animal by TUS. After collapse of the ovulatory follicle on day 1, we observed reduced size of the newly-formed CL (4 mm), with a blood clot in the center Corpora lutea were larger on the following days, and became round by day 4, and reached their mature size on days 6 and 7 (9-12 mm). After recording the ovarian structures, reproductive tracts from both experiments were submitted for oviductal and uterine flushing.

Oviductal flushing technique

The uterine tube ipsilateral to the side of ovulation was dissected from the surrounding tissue in order to stretch the convoluted oviduct. The UTJ and its papillae were also removed. The uterine tube was flushed with 20 ml of phosphate buffered saline solution, containing 10% of fetal calf serum and 10,000 units/ml penicillin and 10,000 mcg/ml streptomycin. A 16 gauge blunt needle connected to a syringe was inserted 2 cm into the uterine tube through the infundibular ostium, and flushed normograde towards the papillae. The flushing medium was recovered in a watch glass and examined under a dissecting microscope for the presence of oocytes.

Uterine horn flushing technique

The uterine horns from females in experiment one were flushed separately starting with the uterine horn ipsilateral to the CL-bearing ovary. In experiment two, both uterine horns were flushed separately. A 16 Fr Foley catheter was gently threaded through the cervix and placed at the base of the uterine horn. The catheter was maintained in place by filling the cuff balloon with 5 to 10 mL of flushing medium. The same procedure was applied to the uterine horn contralateral to the CL-bearing ovary. Each uterine horn was flushed four times with 20 to 60 mL of warm (37°C) flushing medium. Fluid was recovered into a graduated embryo filter and transferred to Petri dishes containing commercial enriched holding medium (ViGro Holding Plus® Agtech, Manhattan, KS), under a 4X and 10X stereomicroscope to determine the presence and condition of the oocytes.

Statistical analysis

Descriptive statistics were used to describe the oocytes on each collection date.

Results

Results from experiment one showed that the interval from GnRH treatment to ovulation was 27.89 ± 1.6 hours (range 26-32 h). The ovulation rate was 83.33% (10/12), and the collection rate was 66.66% (8/12). On day 1 post-ovulation, the two ova collected from oviducts of the two females showed normal morphology, surrounded by the cumulus oophorus. The ova were slightly smaller (150 µ) compared to the ova collected after normal mating (179.47 ± 9.47µ). In comparison the oocytes of llamas ranged from 172 to 200 µ (mean ± SD, 184±14).[10] On day 2 post-ovulation, one ovum collected from two females contained clear, shrunken, and dissociated cytoplasm, and did not display normal cell division. On day 3 post-ovulation, one oocyte collected from oviducts of the two females showed the cumulus oophorus cells. This finding was similar to that observed on day 1 post-ovulation; a completely disorganized and clear cytoplasm. No oocytes were collected from tracts revovered on day 4 and day 5 post-ovulation. On day 6 post-ovulation, two oocytes were collected from the uterine horn ipsilateral to the side of ovulation in two females. The oocytes were shrunken with broken zona pellucida, and a few cells from the cumulus oopohorus were attached to the broken zona. In experiment two, two broken, shrunken and degenerated oocytes were collected from one alpaca with one CL on the left ovary and two CLs on the right ovary. One oocyte was collected from the left uterine horn, and one was collected from the right uterine horn. The other female alpaca had two CLs on the left ovary and two on the right ovary; two oocytes were collected from the left uterine horn and one oocyte was collected from the right uterine horn. The varying degrees of degeneration and disintegration of oocytes collected from the superovulated females were probably a result of different ovulation times and other unknown factors.

Discussion

This is the first study that attempted to determine the fate of the unfertilized ova in alpacas. The interval between GnRH administration and ovulation (27.9 ± 1.6 hours) is similar to the results of previous studies in llamas and alpacas.[10,11] Time of ovulation following administration of GnRH remains quite variable which complicates the timing of oocyte collection based only on the time of administration of GnRH (24 to 36 hours after ovulation induction). The alpaca breeding season in Peru, occurs during the rainy season (December to March) which provides abundant green grass. However, some studies have demonstrated that supplementing alpacas during the dry season when the food supply is low can yield the same results as in the wet season and show the importance of nutrition in regulating the breeding season.[12,13]

Ovulation rates in experiments one and two were 83.33% and 100%, respectively, which confirms earlier reports that high ovulation rates can be achieved in this species with good nutrition and based on ultrasonographic detection of a mature follicle. The overall oocyte collection rates in experiment one and experiment two were 70% and 85%, respectively.

In the current study, oocytes were found in the oviduct from day 1 to day 3 post-ovulation, were at different stages of degeneration, and they were found in the left and right uterine horns at 6 days post-

ovulation with degenerated or with broken zona pellucida. During the oviductal transport of the oocytes, they must be in the area of the isthmus on day 4 and 5 post-ovulation. The isthmus area which is highly convoluted narrows somewhat at the point of entry into the uterus may be the reason that we were not able to collect any oocytes on these days in experiment one or they were blocked at the UTJ. Our results are in agreement with the observation that it is difficult to find embryos at day 6 and 7 after mating.[8,9] Therefore, the collection rate for females on days 4, 5 and 6 post-ovulation may underestimate the actual number of oocytes present in the uterine tubes. Our findings contradict earlier hypothesis that camelids may have selective transport of fertilized ova through the UTJ as seen in the mare.[4]

The results show that unfertilized oocytes reach the uterine horns on day 6 after ovulation demonstrating that there is no selective transport. Therefore, the hatching/hatched embryo may not be needed to open the UTJ. We hypothesize that as the embryo enlarges and develops into a blastocyst, passage through the UTJ is slowed and that the process of hatching from the zona pellucida may be the result of mechanical action of the strong UTJ papilla muscle. Experiment two was performed in order to examine the hypothesis that in superovulated females the first embryo to descend to the uterus sends the signal to open the UTJ and enter the uterine cavity and, as a consequence, the remaining embryos as well unfertilized eggs enter the uterus. However, our results contradicte this hypothesis since unfertilized eggs were found in the uterine horns at day 6 post-ovulation in single ovulating and superovulated females.

We conclude that in alpacas unfertilized eggs are located in the oviduct ipsilateral to the side of ovulation from day 1 to day 5 post-ovulation. They continue their journey and enter the uterine horns on day 6 after ovulation in both single ovulating and superovulated females. The unfertilized oocytes in alpacas are not retained in the UTJ as is the case in mares. We recommend further studies on the mechanism of the oocyte and embryo passage through UTJ in alpacas.

References

1. Fernandez-Baca S, Hansel W, Saatman R, et al: Differential luteolytic effects of right and left uterine horns in the alpaca. Biol Reprod 1979;20:586-595.
2. Fernández-Baca S, Sumar J, Novoa C, et al: Relación entre la ubicación del cuerpo lúteo y la localización del embrión en la alpaca. Rev Inv Pec (IVITA). Univ Nac S Marcos 2, 1973. p. 131-135.
3. Sumar J, Leyva V: Relación entre la ubicación del cuerpo lúteo y la localización del embrión en la llama (*Lama glama*). Anales III Conv. Int. sobre Camélidos Sudamericanos, Viedma, Argentina. 1979. p.124-127.
4. Van Nierkerk CH, Gerneke WH: Persistence and parthenogenetic cleavage of tubal ova in the mare. Onderstepoort J Vet Res 1966;33:195-231.
5. Bouker DA, Kyle CE, McEvoy TG, et al: Superovulatory responses to eCG in llamas (*Lama glama*). Theriogenology 1995;44:255-268.
6. Flood PF, Jong A, Betteridge KJ: The location of eggs retained in the oviduct of mares. J Reprod Fertil 1979;57:291-294.
7. Sumar J: Embryo transfer in domestic South American camelids. Anim Reprod Sci 2013;136:170-177.
8. Sumar J: Studies on reproductive pathology in alpacas {thesis].Uppsala: Swedish University of Agricultural Sciences; 1983.
9. Bravo, PW, Moscoso J, Ordoñez C, et al: Transport of spermatozoa and ova in female alpaca. Anim Reprod Sci 1996;43:173-179.
10. Cárdenas H: Desarrollo morfológico, transporte y supervivencia embrionaria en Alpacas. XX Reunión Científica Anual, Asociación Peruana de Producción Animal, Univ Nac Agraria de la Selva, Tingo María, Perú. 1997. p. 64-66.
11. Del Campo MR, Del Campo CH, Adams GP et al: The application of new reproductive technologies to South American camelids. Theriogenology 1995;43:21-30.
12. Ratto M, Huanca W, Adams JP: Comparison of the effect of natural mating, LH, and GnRH on interval to ovulation and luteal function in llamas. Anim Reprod Sci 2010;91:299-306.
13. Rodriguez C, Arellano P, Torres R, et al: Time interval between natural mating vasectomized mating and GnRH on ovulation in alpacas. ICAR Proceedings Satellite Meeting on Camelid Reproduction, Vancouver, Canada 2012. p. 15-18.