A039E TAI/FTET/AI

Comparison of INRA96 and Andromed as an extender for alpaca epididymal spermatozoa

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Keywords*:* alpaca epieidymal sperm, Andromed, INRA96.

Breeding animals by artificial insemination rather than by natural mating has many advantages, for example, to prevent the spread of infectious disease and to allow males of superior genetic merit to produce offspring from a large number of females. However, the technique of artificial insemination is not well developed in alpacas for several reasons, one being the difficulty of working with the viscous ejaculate. Thus, it is difficult to develop protocols for semen handling, including choosing a semen extender. A first step in the development of such a protocol could be to use epididymal spermatozoa to test semen extenders. Two commercial semen extenders, Andromed (A; Minitüb; Tiefenbach, Germany) and INRA96 (I; IMV Technologies, L´Aigle, France), were chosen for this study. Neither of these extenders contains material of animal origin. Objective: to compare the two semen extenders for their suitability for alpaca epididymal spermatozoa. Materials and methods: scrotal contents were obtained from castration of males (n=10) for husbandry purposes. After removal from the animal, the organs were placed in a plastic bag containing phosphate buffered saline and were sent overnight to the laboratory at the Swedish University of Agricultural Sciences (SLU) in a Styrofoam box with a cold pack. This type of packaging is used to transport stallion semen and maintains the temperature at approximately 6 ºC overnight. The tunica vaginalis, connective tissues and blood vessels were removed; after isolating the cauda epididymis from the testis, it was placed in warm (37ºC) semen extender. From each animal, one cauda epididymis was placed in A and the other in I; several cuts were made in the epididymis to allow the contents to flow out. After incubation for 10 minutes at 37°C, sperm motility was measured by computer assisted sperm analysis (CASA; SpermVision, Minitüb), membrane integrity (MI) was assessed after staining with SYBR14/propidium iodide (Live-Dead Sperm Viability KIT LIT L-7011; Invitrogen, Eugene, OR, USA), and acrosome status was determined by staining with FITC-conjugated peanut agglutinin (Sigma, St. Louis, USA). The CASA analysis was repeated incubation for 30 minutes. Means were compared by mixed model using the SAS® software (version 9.3); significance was set to $P \leq 0.05$. Results: LSMEAN (± SEM) after 10 minutes for A and I, respectively, were as follows: total motility 19±5% vs. 21±5% (not significant, NS), MI 58±9% vs. 56±9% (NS); intact acrosomes 65±7% vs 54±7% (NS). Total motility in A and I after 30 minutes was 29±4% and 35±4% (NS), respectively. Progressive motility in I after incubation for 30 minutes was 12±4% compared to 25±4% after 10 minutes ($p<0.05$). However, progressive motility in A was not different between the two time points (11±4% vs. 17±4%, respectively). Conclusion: viable epididymal spermatozoa could be obtained from the material even after overnight transport. There were no differences between the two extenders in the sperm parameters evaluated. Therefore, either extender could be used for alpaca spermatozoa. And should be tested in an insemination trial.

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040E TAI/FTET/AI

Influence of parturition number of the recipient on an embryo transfer programme in wool type ewes

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Keywords*:* ewes, ovulation rate, pregnancy.

Embryo transfer is important for the multiplication and rapid propagation of sheep breeds of high genetic merit. The selection of the most appropriate genotypes of recipient ewes is essential to obtain high pregnancy rates. The objective of this study was to evaluate the fertility of two groups of ewes (nulliparous and multiparous) exposed to an embryo transfer program. The study was conducted from January to February of 2019 at the commercial sheep farm "Poza Rica", which is located in a temperate area named Singuilucan, in central Mexico. A total of 142 healthy and good body condition Hampshire ewes were used, from which 97 were nulliparous and 45 multiparous. The ewes were synchronized with intravaginal sponges containing 20 mg of micronized cronolone (Chrono Gest, Intervet, Netherlands), which were inserted for 12 days. On day 10, the ewes were injected intramuscularly with 400 IU eCG (Novormon, Sanfer, Mexico). The estruses were detected every 6 h with two fertile Kathadin rams equipped with an apron, starting 18 h after sponge removal. The time of estrus was recorded. On day 6 after estrus detection, just before embryo transfer, ovulation rate was determined by laparoscopy. The recipients received an embryo of transferable quality (compact morula or blastocyst) within 3 h after its collection, coming from Dorper donor ewes using a laparoscope and standardized procedures. The embryos were kept in holding medium (Syngro, Vetoquinol, Canada) were transferred using a capillary glass tube in the ipsilateral horn to the ovary in which ovulation was recorded, and the presence of the best quality corpus luteum was determined based on its size. On day 35, pregnancy diagnosis was carried out using an ultrasound machine and a 3.5 MHz transabdominal probe (Aloka Prosound 2, Japan). The results of the incidence of estrus and pregnancy rate were analyzed as categorical variables with the GENMOD procedure, and ovulation rate with the GLM procedure, both of them available in SAS. All the ewes were detected in estrus in both treatments. The incidence of estrus was different ($p<0.05$) for ewes from nulliparous and multiparous at 24 h (62.9 and 55.5%), 30 h (32.9 and 45.4%), and 36 h (4.1 and 0%) after sponge removal. Ovulation rate was higher ($p<0.05$) in multiparous than nulliparous ewes (2.11 ± 0.12 vs 1.76 ± 0.08). Moreover, the pregnancy rate was lower ($p<0.01$) in nulliparous than multiparous ewes (29.9 vs 68.9%). In conclusion, under the conditions of the study, the results showed the feasibility of using multiparous Hampshire ewes as embryo recipients.

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Effect of age of the recipient on an Embryo Transfer programme

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Keywords*:* ewes, synchronization, embryo.

The reproductive technique of embryo transfer has been used to intensively reproduce high genetic merit animals of several species in different countries. The success of the technique relies on the control of every step to achieve high pregnancy rates. The objective of the study was to evaluate the fertility of two groups of ewes (nulliparous and multiparous) subjected to an embryo transfer program. The study was conducted from November to December of 2018 at the commercial sheep farm Rancho Poza Rica, which is situated in Singuilucan, Hidalgo, Mexico including a total of 46 ewes, from which 20 were nulliparous Katahdin ewes (T1) and 26 were multiparous Katahdin ewes (T2). The ewes were healthy, in good body condition (3.0) and were synchronized with intravaginal sponges containing 20 mg micronized cronolone (Chrono Gest, Intervet, Netherlands), inserted for 12 days. On day 10, the ewes were intramuscularly treated with 400 IU eCG (Novormon, Sanfer, Mexico). The estruses were detected every 6 h with two fertile Pelibuey rams equipped with an apron, starting 18 h after sponge removal. The time of estrus was recorded. On day 6 after estrus detection, just before embryo transfer, ovulation rate was determined as a number of corpora lutea observed in ovaries during laparoscopy. The recipients received one tranferable compact morula or blastocyst within 2 h after embryo recovery from a Charollais donor ewe using laparoscopy. The embryo recovered into holding medium (Syngro, Vetoquinol, Canada) was transferred using a capillary glass tube in the ipsilateral horn to the ovary in which ovulation was recorded or the presence of the best quality corpus luteum was observed, determined on the basis of its size. On day 45, pregnancy diagnosis was conducted using an ultrasound machine and a 3.5 MHz transabdominal probe (Draminski Animal profi 2, Poland). The results of incidence of estrus and pregnancy rate were analyzed as categorical variables with the Proc GENMOD function and ovulation rate with the procedure Proc GLM, both of them available in SAS. It was considered $\alpha 0.05$ to estab lish significant differences between treatments. The general percentage of estrus was similar for ewes from T1 and T2 (76.9% vs 100%). The incidence of estrus was different ($p<0.05$) only for ewes from T1 and T2 at 24 h (50% vs 90%), but similar ($p>0.05$) at 30 h (19.2% vs 10.0%) and 36 h (7.7% vs 0%). Ovulation rate was higher in ewes from T2 compared to ewes from T1 (2.0 ± 0.22 vs 1.27 ± 0.13). Furthermore, 50% of the ewes were pregnant in both treatments. In conclusion, the results showed the feasibility of using Katahdin ewes as embryo recipients regardless of their age and parity.

A042E TAI/FTET/AI

Import of Belgian Blue embryos in tropical Indonesia: birth of first calves

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Keywords*:* Belgian blue cattle, Indonesia.

The Belgian Blue breeding program has been started to improve local beef production in Indonesia. Belgian Blue cattle (BB) are characterized by a double-muscled phenotype caused by a deletion within the myostatin gene. Animals present less bone and fat, more muscle, as well as a higher muscle bone ratio than other breeds (Kolkman et al., Reprod Domest Anim 47, 365, 2012). Indonesia is Southeast Asia's biggest economy, and its population growth, rising incomes, and changing public tastes, caused the beef consumption in this country to increase over time (increase of 4.66% per year), while the growth of domestic beef production is only 3.20 % per year. As a result, beef import rose by 21.58% annually (Kusriatmi et al., Journal of the ISSAAS 20, 115-130, 2014). One of the efforts that the Indonesian government did was importing BB frozen semen and embryos from Belgium into Indonesia (Agung and Syahruddin, 16th AAAP Animal Science Congress 2, 10, 2014). Consequently, the first BB calves were born in South Asia following successful embryo transfer (ET). The result of BB born by ET is 94, with the total of pregnant cow is 138 from 588 of total pregnant checked by rectal palpation; while the result of BB born by artificial insemination (AI) is 168, with the total of pregnant cow is 278 from 545 of pregnant checked by the same method (Indonesian Animal Husbandry, 2019). From those result, we could find that the successful percentage of AI is higher than the ET application with 51% and 23% respectively. Furthermore, all of BB calves by AI was born by normal parturition, while the BB calves by ET was born by C-section. In order to follow up this program and to predict the future of BB in this tropical environment, we compared the birth weight of BB pure breed in Indonesia (by ET) with the crossbreeds (by AI with several Indonesian local cattle) with a total sample size of 105 calves. Furthermore, we also compare the birth weight of BB calves born by ET in Indonesia versus calves born in Belgium, with a total sample size of 115 new born calves. The results indicated that the mean of BB pure breed birth weight in Indonesia 51,23 kg is higher rather than the crossbreed of BB with Friesian Holstein, Simmental, Limousine, Peranakan Ongole, Angus and Madura; with their mean of birth weight 44,80 kg; 43,5 kg; 36,14 kg; 29,59 kg; 46,6 kg; and 25,5 kg respectively. In addition, the result of birth weight of BB in Indonesia versus in Belgium showed that there are a significant different in their birth weight ($\alpha = 0{,}042 < 0{,}05$), which the mean of BB birth weight in Belgium (52,39 kg) is higher rather than the mean of BB birth weight in Indonesia (52,00 kg). This significant difference might be related to the tropical condition in Indonesia. Based on (Brody S, Journal of Dairy Science 39, 6, 715-725, 1956), the environmental comfort zone for European cattle ranges between -1 and 15℃, while the temperature in Indonesia is on average 18°C-30°C (Indonesian Directorate of Animal Husbandry, 2019). In addition, the BB is assumed to be more susceptible to heat stress than most other breeds, owing to the reduced oxygen transport efficiency, caused by the relatively small volume of their heart and lungs in comparison with their body volume (Grobet et al., Mammalian Genome 9, 210, 1988).