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Dear Colleagues,

We are pleased to formally present the Proceedings of the VI Peruvian Congress Animal Reproduction of the Asociación Peruana de Reproducción Animal (ASPRA). We hope you enjoy the meeting and take advantages of the opportunity to gain new scientific insights, renew friendships and make new contacts. The organizers are pleased with SPERMOVA editors and staff for the support of included abstract of our congress. Our goal of this publication of abstracts in English Language, is to encourage students and researchers the adoption of English as the universal language of science. Similar to the previous year, this event was planned considering both the Organizer Committee along with the members of Scientific Committee has brought together diverse topics and speakers to stimulate thoughts and discussion. In addition to the traditional plenary, we will have roundtables to discuss relevant issues are also part of the program. We also want to thank all the speakers who have agreed to attend this meeting and share their knowledge with us. My special thanks for all ASPRA Board and collaborators, whom have turned this meeting in to a reality

Kind regards

Juan Reategui, PhD
President
(2016-2017)

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{1} INFLUENCE OF THE AGE MALE ON THE FUNCTIONALITY IN RAW ALPACAS SEMEN

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ABSTRACT

The age of the male reproductive alpacas is 4 to 8 years and there is evidence in other species that the age of the male sperm can affect certain characteristics negatively. The functionality of the sperm membrane is essential for sperm to fertilize, which is evaluated by endosmótic response sperm in semen camelids best answers hypoosmotic 100 mOsmol solution was found. The vitality is evaluated using supravital staining as Nigrosine-eosin, which has been used in semen camelids. Sperm motility can be assessed by measuring the progressive advance speed. The aim of this study was to determine the effect of the age of the animal on the endosmótic response, sperm vitality and speed in fresh semen of male alpacas 4, 6 and 8 years old. 12 adults males of proven fertility, 4 animals distributed according to age into three groups, whom I were trained for semen collection by artificial vagina technique using a dummy during breeding season were used. 5 collections of semen from each animal was obtained twice a week, making a total of 60 ejaculates evaluated. All semen samples were treated mechanically by repeated sample through a syringe with the aim of reducing the viscosity passage. Endosmosis assessment was performed by incubation of semen for 30 min in a hypoosmotic solution (100 mOsmol). The vitality was evaluated by supravitally eosin-nigrosine staining and sperm velocity was evaluated by measuring the time required for a sperm crossing a box of 50 μm in a Neubauer chamber. Statistical analysis was performed using a completely randomized design with subsamples and Tukey test for comparison of means; the relationships between variables and age of the animals were evaluated by correlation, regression and coefficient of determination. The endosmótic response (59,8, 61,8 and 58,2%), vitality (58,85, 60.35 and 58.35%) and sperm speed (21,7, 28,4 and 58,2 µm/s) To 4.6 and 8 years respectively had values found within the range described for the species. There is statistical difference between the three tested ages ($P \leq 0,01$), however no significant difference between subsamples. Correlations (-0,394, -0,408 and 0,183) regressions (-0,37, 0,13 and 1,19) and the coefficient of determination (0,156, 3,4 and 16,6) respectively for endosmosis, sperm vitality and speed were respectively low and insignificant so the influence of age evaluated not seem to be so important. The feature that suffers biggest drop in percentage sperm is speed. These results are similar to other species where the seminal characteristics decrease with the age of the individual assessed on the seminal characteristics evaluated, so we might assume that the males used between these ages would show sperm with good characteristics, being optimal semen of male 6 years.

Keywords: sperm membrane, alpaca, semen, age

{2} ENDOMETRIAL CYTOLOGY AS AN INDICATOR OF SUBCLINICAL ENDOMETRITIS OF DAIRY CATTLE, HOLSTEIN FRIESIAN AND JERSEY BREEDS

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ABSTRACT

In order to evaluate the presence of polymorphonuclear neutrophils (% PMN-N) as an indicator of endometritis sub clinic in dairy cattle Holstein Friesian and Jersey breeds, by the method of endometrial cytology. 94 dairy cows were sampled, and were grouped by genotypic characteristics as: Group 1: 47 Holstein Friesian cows; Group 2: 47 Jersey cows, both between 21 and 56 days postpartum. It were evaluated: age, body condition, lactation number, number of birth, date of birth and days in milk to obtain the sample data were evaluated with a test of homogeneity based on statistical Chi square ($p < 0,05$). From each cow a cytological sample of the endometrial mucosa was took, using adapted cervical brushes. Smears were air dried and set, then were taken to the laboratory to be colored by Diff-Quick staining, to cell reading fields proceed. The neutrophils were used to determine the degree of inflammation of the uterine lining, obtaining a percentage of Polymorph Nuclear neutrophils (PMN-N %), relative to the total cells. The criteria for diagnosing of positive subclinical endometritis (SE) was ≥5,10% of PMN-N (Reátegui, *et al.*, 2015) in each smear. The frequency of subclinical endometritis in cows of different genotype did show statistically significant differences ($p< 0,05$), it is observed that 59,57% of Holstein Friesian cows under study are positive to subclinical endometritis compared to 27,66% of Jersey cows having a marked percentage of SE difference presentation. The frequency of SE of both breeds as lactation, showed that in the 2nd and 4th-feeding showed statistically significant differences ($p <0,05$). Also it shows that 53,8% of Holstein cows with 2 lactations are positive to subclinical endometritis compared with 11.1% of Jersey cows. Similarly it is observed that 75,0% of the Holstein Friesian 4 lactations are positive subclinical endometritis against any case Jersey cows. The frequency of subclinical endometritis of both breeds by days in milk showed that cows with 34 - 46 days in milk showed statistically significant differences ($p <0,05$). It also shows that 64.0% of the Holstein Friesian cows with 34 to 46 days in milk are positive to subclinical endometritis compared to 23,1% of Jersey cows. Garofolo, *et al.,* 2013 noted that between the different genetic groups in primiparous cows no significant differences ($p>0,5$) was found in any of the variables studied, the% PMN-N reached a range between 0,4% and 4,4%, with an average of 2,2% still below the values indicating the present investigation reports the% PMN-N by genetic group both as multiparous or primiparous cows showed no significant differences between them. It has be concluded that the overall frequency for SE in different genotype cows did show statistically significant differences ($p>0,05$), however the presence of PMN-N as an indicator of subclinical endometritis in dairy cows of different genotype with 2 and 4 lactations showed differences statistically significant ($p<0,05$).

Keywords: Prevalence, subclinical endometritis, polymorphonuclear, postpartum

{3} REPROGRAMMING CELL CAPACITY OF COWS CREOLE EGG FOR GENERATING CLONES MADE BY HAND IN CATTLE CLONING: PRELIMINARY RESULTS

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ABSTRACT

For two decades has evaluated different types of cells is their potential use in producing successfully cloned by somatic cell nuclear transfer, but a very little attention in effect of oocyte. The aim of this study was to evaluate the cellular reprogramming capacity of oocytes creole cows for producing cloned cattle using a practical procedure of somatic nuclear transfer, known as Hand Made Cloning (HMC) (Vajta *et al.*, 2003. During this preliminary study, 773 cumulus-oocyte complexes (COCs) were obtained from slaughterhouse ovaries of creole cattle and matured in four wells plates (25 to 30 oocytes per well) for 21 hours at 38,5° C in 5% CO2 atmosphere, in TCM199 medium supplemented with 0,6 mM glutamine, 0,2 mM pyruvate, 0,01 IU/Ml LH and FSH, 1 µg/mL estradiol, 50 µg/mL gentamicin, 10 ng/ml EGF and 10% FCS. Subsequently, oocyte maturation were evaluated by expansion of the cumulus cells and the presence of the first polar body. The nude oocytes are incubated in demecolcine (2,5 µg/mL) in IVM medium for 2 h to promote the formation of the cone with the metaphase plate to guide the manual enucleation. The zona pellucida was removed by incubation for 3 min at pronase 2mg/ml. Enucleation was performed manually with a microblade, producing 252 hemi-cytoplasts. The fusion of citoplast and a fibroblast was performed by method "sandwich" with electrofusion BLS (Budapest, Hungary) and BTX fusion chamber 0,5mm (BTX Corp., San Francisco, CA, USA). Fibroblasts from posterior auricular were cultivated in vitro to pass3 with a donor nucleus (somatic cell). The fused cytoplast were chemically activated by incubation for 5 minutes at room temperature in 7% absolute ethanol in TCM Hepes supplemented with 20% FBS, followed by 5 hours incubation at 38,5 ° C in 5% CO2 atmosphere, in TCM199 medium with 5 µg/mL citocalacina B and 10 µg/ml cycloheximide. Embryos were cultured in bags with gas mixture 90% N_2, 5% O_2 and 5% CO_2 in SOFmodify medium. At day 7 of culture the percentage of blastocysts produced were evaluated. As a result 77,3% of oocyte maturation (598/773), 86% of successful fusion of reconstructed embryos (91/105) of which 25 embryos developed to blastocyst stage (27,9 ± 10,1%) was obtained. Thus it was demonstrated that oocytes from creole cattle are able to efficiently generate cloned cattle embryos.

Keywords: Cattle, cloning, electrofusion, blastocyst.

{4} REPRODUCTIVE PARAMETERS OF DIFFERENT DAIRY CATTLE GENOTYPES IN THE ECUADORIAN AMAZON

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ABSTRACT

The strategies of crossbreeding, selection and improvement in systems of bovine cattle production in the tropics, must be set taking into account the agroecosystem, aiming to achieve efficiency in the sustainable production of the system. The objective of this work was to evaluate the reproductive behaviour of four milking genotypes of first lactancy, corresponding to the years 2014-2015. It was evaluated the dynamic behaviour of reproductive variables of the cross milking cows, of four genotypes: Brahman x Brown Swiss (BS) n=12, Brahman x Jersey (J) n=10, Brahman x Sahiwal (S) n=8 y Brahman x Gyr (Gyr) n=6 belonging to the milking rodeo of the Centre of Investigation, Postgrade and Conservation of Amazonian biodiversity (CIPCA). The centre is located in the ArosemenaTola canton, in the province of Napo, kilometre 44 via Puyo-Tena (coordenates: S 01° 14.325´;W077° 53.134´). It has a tropical climate with 4000 mm/year rainfall, an average relative humidity of 80% and temperatures which range from 15 to 25°C. The reproductive data came from the periodic controls carried out by a veterinarian consultant responsible for the CIPCA. The cows were always inseminated with tested bulls. They were fed on free pasturing. The variables under analysis were: age at first delivery in months (EPP), first delivery - first oestrus interval (IPPC), birth – conception interval in days (IPC), delivery – delivery interval in days (IPP). The average figures and the standard errors were estimated for all the measured variables. The statistical analysis was carried out using the variance analysis with a criterion of classification and multiple comparison tests HSD by Turkey-Kramer HSD ($p \leq 0.05$). IPPC (days): BS (78 ± 8 a), J (77 ± 8 a), S (78 ± 10 a), Gyr (66 ± 12 a); IPC (days): BS (228 ± 32 a), J (219 ± 18 a), S (162 ± 34 a), Gyr (215 ± 64 a); IPP (days): BS (509 ± 32 a), J (500 ± 18 a), S (443 ± 34 a), Gyr (496 ± 64 a). There are significant differences ($p \leq 0.05$) as regards age at first deliveryBS (37,1±1), J (39,7±1), S (42,6±1) andGyr (41,4±1) months. Such numbers are high if compared with the 35,4±5 months cited by other authors. There are no significant differences as regards the rest of the variables ($p \geq 0,05$) among the genotypes. Figures of 452±100 in days of IPP have appeared in double-purpose cows based on the crossbreeding zebu and Holstein which is similar to the figures found in this work. The average numbers of IPPC for the four genotypes are within the ideal figures desired for milking cows in milder environments either from the nutritional as well as from the climatic point of view. The S cows show a better reproductive tendency than the rest of the genotypes and it is the oldest one when reaching first delivery. This might let them face reproduction in a different way. It could be concluded that the genotypes under study show a similar reproductive behaviour at first delivery.

Keywords: Dairy cows, genotypes, reproductive index, grazing systems

{6} INFLUENCE OF EFFECT OF PREGNANCY AND LACTATION ON ALPACA FOBER DIAMETER IN ESTIMATED BREEDING VALUES

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ABSTRACT

The accuracy of estimates of genetic values in the genetic improvement program in alpacas depends on the estimates of the residual variance is as small as possible, for it to know the effects that can influence on the diameter of the fiber, and that these may be included within the model is important, one of the effects included is sex, this, in populations with no selection has been shown to have not relevant differences, but females to go through physiological stages that require energy expenditure as pregnancy and lactation may have an influence on the diameter, that is why this paper aims to estimate the effect of gestation and lactation on the diameter of the fiber, to better define the genetic values. This has been analyzed 10,983 data stored in the PacoPro v5.2 from 2000 to 2015 in Pacomarca genetic center, corresponding to 8,744 animals, 6,899 Huacaya (HU) and 1,845 Suri (SU), analyzed the data separately for ecotype, for estimate the effect of energy expenditure have been grouped into five categories, Male (MV) and empty females non-lactating (VNL) that have no energy expenditure pregnancy or lactation and groups of pregnant non-lactating (PNL), pregnant lactating (PL) and empty females lactating (VL); to avoid bias diameter of VNL at an early age, it has been included reproductive age in years at 06 levels from the third year and has grouped all over 8 years on one level; the age at fiber analysis has been included as a covariate linear and quadratic in days. In Table 1, the mean (μm) and its standard error, Multi-factor ANOVA shows that here is significant difference ($p<0.05$) in the effects of energy expenditure and energy expenditure in interaction with reproductive age are shown HU, but there is no significant difference ($p> 0.05$) for the interaction energy expenditure - reproductive age for SU, we used the method of Duncan for the multiple comparison factor levels of energy expenditure. The differences may be due to hormonal changes and females prioritize the mobilization of nutrients and reserves milk production, followed by the development of pregnancy. Can finally conclude that there effect of gestation and lactation on alpaca fiber diameter, and this can be included from the third year of life and energy expenditure within the model to refine estimates of genetics values.

Table 1. Physiological factors affecting the fiber diameter in alpacas

Category	Huacaya			Suri		
	Records (n)	Diameter (μ)	Sig	Records (n)	Diameter (μ)	Sig
MV – Male	886	26,14	c	312	27,88	c
VNL- Females non-lactating	1454	24,39	a	310	27,52	bc
VL- Empty females lactating	1360	24,34	a	359	27,02	ab
PNL- Pregnant non-lactating	1529	25,44	b	437	27,60	bc
PL- Pregnant lactating	3360	24,47	a	976	26,76	a

Keywords. *Alpaca, fiber, gestation, lactation, genetic value*

{7} SOME SEMINAL CHARACTERISTICS BULLS OF MEAT EUROPEAN RACE BRED IN TERMS OF HEIGHT A 3,900 m.s.n.m.

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ABSTRACT

With the development of artificial insemination in cattle and increasing the genetic value of animals it has increased recognition of the importance of examining the reproductive fitness of the bulls before they are put into service or are intended for collection and preservation of semen in artificial insemination centers; however, at present research it is lacking regarding semen characteristics of bulls meat production especially in extreme height. The objective was to evaluate the seminal characteristics of two breeds of bulls European meat obtained pure by crossing the Peruvian altiplano conditions. The research was conducted at the research center and production Chuquibambilla, Universidad Nacional del Altiplano located at an altitude of 3900 meters, with four bulls (two angus race and two race Charolais) aged between two and three years whose weights ranged from 465 and 535 kg; the animals were kept under extensive management fed on natural pastures improved with alfalfa (Medicago sativa) associated with orchardgrass (Dactylis glomerata). After a workout in semen collection, semen samples were obtained using an artificial vagina (IMV, France) for 10 months from April 2015 to January 2016 with a frequency of two collections per month. Seminal the following characteristics were evaluated: volume (ml) determined sperm concentration (number of spermatozoa per ml of semen) using a Neubauer chamber and evaluated on individual motility Total heating stage at 37 ° C under microscope at 100x. 28 Freshly collected semen samples for each race, the same as immediately placed in a water bath at 37 ° C for evaluation were evaluated. The statistical analysis of the data was performed by Student t test for independent samples at a significance level a = 0,05 using the statistical package Minitab 16. Prior to statistical analysis data volume and sperm concentration They conducted tests of normality and homogeneity of variance, whereas motility was subjected to a transform of the square root of the arc sine proportional percentage value. The results of volume average 2,8 ± 0,7 ml Bulls beat Aberdeen Angus breed (p = 0,043) to the Charolais breed that reached 2.4 ± 0.6 ml; sperm concentration showed no statistical difference (p = 0,244) between the two genotypes being 883 ± 159 and 839 ± 118 million sperm / ml in Aberdeen Angus and Charolais, respectively; total sperm motility was 76.4 ± 5,6 Variable and 76,4 ± 5,.7% in Aberdeen Angus and Charolais, respectively, the same as showed similarity (p = 0,351). In conclusion, seminal characteristics of beef breed bulls (Aberdeen Angus and Charolais) reared in the Peruvian highlands, have good characteristics for use in programs sperm cryopreservation and artificial insemination.

Keywords: Feature seminal, Aberdeen Angus, Charolais, altiplano

{8} EFFECT OF THE BUTILHIDROXITOLUENO (BHT) AND SEMINAL PLASMA IN SHEEP CRIOPRESERVACIÓN OF SPERMS

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ABSTRACT

During the process of semen cryopreservation, levels of reactive oxygen species increase significantly, with losses of sperm quality and increased premature sperm capacitation, one way to reduce these effects is by adding components with antioxidant capacity freezing the diluent. Among them, butyl hydroxytoluene, has been successfully tested in different species. The objective of the study was to evaluate the effect of supplementation Butylhydroxytoluene (BHT) (0.6 mM, 2.0 mM and 5.0 mM) and presence / absence of seminal plasma (PS) during ram semen cryopreservation. There were used 48 ejaculated ones of 8 rams (4 Xisquetas and 4 Aranesas) of 2 years of age like donors of semen, that 2 times were collected by artificial vagina for week, during the autumn station. After collection, the ejaculates were mesclaron in a single tube (pool) then divided into two aliquots. An aliquot PS (washing) was removed, to which diluted (1: 5) with 0,3 M Tris, 27,75 mM Glucose and 94,7 mM Citric Acid (TGC), and centrifuged at 600g at room temperature for 10 minutes two times while the other aliquot was not washed, to obtain the PS, part of fresh semen (pool) was used, for which, centrifuged at 10000g/10minutes, 5 ° C twice. Aliquots of washed and unwashed sperm are diluted with TGC 5% glycerol and 15% of egg yolk powder supplemented or not with BHT and/or PS resulting eight treatments: (L: Semen washing and diluent, L + 0,6 BHT: L with 0,6 mM BHT, L + 2,0 BHT: L with 2,0mM BHT, L + 5.0 BHT: L with 5,0 mM BHT, L + PS: L with 13% (v/v) of seminal plasma, SL: Semen not washed and diluent, SL + 5,0 BHT: SL with 5.0 mM BHT, L + 5,0 BHT + PS: L with 13% (v/v) of seminal plasma more 5,0 mM BHT). The samples were subsequently of semen were cooled to 5 °C for 4 hours to be packaged in pajuelas 0,25 mL to $400x10^6$ concentration of sperm/mL and frozen for 10 minutes in liquid nitrogen. Motility, viability and HOST were determined at the defrosted. To determine the integrity and functionality of the plasmatic membrane used the fluorocromos SYBR14 and ioduro of propidio (IP), the integrity of the membrane acrosomal the lectina Arachis hipoge (PNA) with Ficoeritrina (PE) and the activity mitocondrial with Mitotracker deep network, all the readings were realized by means of citometría of flow. Data were analyzed using GLM (ANOVA) procedure of SPSS 20, with 6 repetitions in each treatment and Bonferroni test. Comparison of different concentrations of BHT showed that treatment L + 5,0 BHT independent of the addition of PS in diluents, provided the best ($P < 0,01$) values in viability (46,3 ± 0,6%), HOST (42,9 ± 0,5 %) total motility (45,7 ± 1,1%), progressive motility (29,0 ± 0,7%) and live with intact acrosome and mitochondrial activity (43,3 ± 0,4%) in defrosted compared to other treatments. With significantly inferior values ($P < 0,05$) in the SL and intermediate values in the other treatments. The addition of 5 mM BHT, regardless of the presence of PS, gave the best quality parameters seminal defrosted. Also, sperm washing had a beneficial effect on sperm cryopreservation. However, supplementation with 13% seminal plasma media cryopreservation of sperm washings had no effect on semen quality parameters.

Keywords. Butilhidroxitolueno, seminal plasma, cryopreservation, sperm, ovine.

{9} RELATIONSHIP BETWEEN PHYSICAL CHARACTERISTICS AND IONIC CONTENT OF CERVICAL MUCUS PREGNANCY STATUS TO INSEMINATE HEIFERS DETECTED IN ESTRUS

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ABSTRACT

Heat detection is usually one of the many factors that affect pregnancy rate in dairy farms, inseminating females outside the optimum time to achieve fertilization. The aim of this study was to analyze the macroscopic characteristics (size, appearance, and consistency), crystallization phenomenon, pH, level of calcium, magnesium, potassium, sodium and chlorine in the cervical mucus of heifers in order to relate these properties with the periovulatory period. 20 Holstein heifers were used. Mucus was obtained before insemination and was collected by suction from the cervix by syringe (60 ml) connected to sheath insemination. It was determined pH using reactive tape (6 to 7,9) and a drop of mucus extended performed, allowed to air (30 minutes) and the degree of crystallization (0 - 4) was evaluated according to typical and atypical formations fern leaves. Then the samples were stored at -20 ° C until further use. The ion content was determined using commercial kits (Wiener Laboratories SAIC) and the concentration of each ion is expressed in milliequivalents per liter. Pregnancy was detected at 60 days after insemination. The variables studied were pH, degree of crystallization, concentration of Na, K, Ca, Mg and Cl and Na / K ratio was calculated. Each variable was described by its mean and standard deviation and through an ANOVA significant differences between means (-P- pregnant and empty -V-) was established. The results of this study demonstrated that pregnancy was associated with mucus containing a significantly lower concentration of K and Mg, compared with empty heifers (K, P: 7,76 V: 12,97; Mg, P: 2,80 V: 3,93; $p<0,05$ $t = 2,16037$). For Na, Ca and Cl were no significant differences (Na, P:140,62 V:134,57; Ca, P:2,30 V:3,77; Cl, P:189,14 V:199,83; $p<0,05$ $t=2,16$). Also pregnancy was associated with a significantly higher for ion ratio Na/K calculated (Na/K P: 18,40 V: 12,16; $p <0,05$ $t=2,16$). In-pregnant heifers the pH was significantly higher, and the degree of crystallization significantly lower (pH, P: 7,60 V: 6,70; Crystallization P: 1,31 V: 2,21, $p<0,01$ $t=3,01$). The results show that the optimum time to inseminate was associated with a cervical mucus is observed macroscopically fluid, transparent and abundant, with a pH above 7,0 and crystallized fern leaves forming atypical, mostly with average grade of 1,31. Probably the fluid or liquid consistency remains regarding the result of a higher ratio of Na/K ions may exert active osmotic force, responsible for water retention secretion, likely favoring the transport of sperm needed for fertilize the egg and then achieve pregnancy.

Keywords: *Cervical mucus, estrus, ionic content, pregnancy.*

{10} POSSIBLE FACTORS AFFECTING PREGNANCY RATE OF COWS IN THE AMAZON ECUATORIAN

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ABSTRACT

The objective of this work was to assess how the cow pregnancy depends type artificial insemination, breed and follicular size in cattle with dual purpose in the Ecuadorian Amazon. The 341 records of the individual services were evaluated from January 2015 to December 2015 that corresponds to cows of different genotypes and cross: Brown Swiss (BS) n = 135, Charolais (Ch) n = 24, Holstein Fresian (HF) n = 104, Jeresey (J) n = 12, Normando (N) n = 21, x Brown Swiss Holstein Friesian (HFxBS) n = 35 and Holstein Fresian x Normando (HFxN) n = 10. The work was conducted in the Cantons and Santa Clara which is located in the Pastaza Province (Ecuador). During the practice was taken in count the following criteria: (1) There were used exclusively cows with body good condition ≥ 2.5 (scale 1-5), (2) the same technical inseminator was used to inseminate the all cows, (3) gynecological checks were previously of the protocol that determinates the viability of the cow`s reproductive system , (4) the same protocol was defrosted, (5) the semen that was used, it contains the quality that fulfilled with the requirements of quality that have to be used, (6) the cattle are free of brucellosis, tuberculosis, campylobacteriosis and trichomoniasis; with control of leptospirosis, IBR and BVD and data reliability. The Two insemination techniques were performed: a estrus detected (natural) 136 cows and a Insemination Time (TAI) that follows the protocol: Day 0 CIDR vaginal device 1.9 gr. progesterone plus the application of 2 mg estradiol benzoate, and removal the device after the 7 days, plus the application of 25 mg of prostaglandin (PG2F - Pfizer) and 400 IU of eCG plus application of 0,5 mg estradiol cypionate . TAI was performed 52 to 56 hours and removed the vaginal device with a dose of 2,5 ml of GnRH. For both techniques the follicular development in the proestrus of all cows were measured by ultrasound. The pregnancy diagnosis was performed by ultrasound during 45 days after insemination. A linear generalized model Logistic of regression was used by JMP statistical software in ITS version 5.0 for Windows (JMP®, SAS Institute, 2003), that was considered the dependent variable: "Pregnancy" which has two categories "Pregnant" or "Nonpregnant". The independent variables are "breed" (BS, CH, HF, J, HFxBS and HFxN), the "Technical Service" (Natural and IATF) and "Follicular dominant size" (DF). An additive model was adjusted and it was not significant for the interactions between independent variables and the result of the model. It was found that the variable DF is the only that contributes significantly to the model. That is, pregnancy is significantly affected by follicular development (p-value <0.0001), but is not related to the technical service (p-value = 0.3412) and breed (p-value = 0.1656). Maximum Credible estimates the coefficients model and calculated odds ratios. For every unit increase in the value of follicular development, the chance of "pregnant" is 14 times higher than "nonpregnant". Keeping fixed the categories for breed and service. To conclude that there was no difference between breed and insemination technique that was used, even if a relationship between follicular size in proestrus and pregnancy in the Ecuadorian Amazon.

Keywords: Cows, genotypes, insemination techniques, grazing systems

{11} DIAGNOSTIC IMPACT OF SUBCLINICAL ENDOMETRITIS ON REPRODUCTIVE PERFORMANCE OF HOLSTEIN FRIESIAN AND JERSEY BREEDS

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ABSTRACT

In order to evaluate the presence of polymorphonuclear neutrophils (% PMN-N) as an indicator of subclinical endometritis and its impact on reproductive performance in dairy cattle Holstein Friesian and Jersey breeds by the method of endometrial cytology, 94 dairy cows were sampled, and were grouped by genotypic characteristics in 47 Holstein Friesian cows and 47 Jersey cows, all between 21 and 56 days postpartum. The variables evaluated were: First Interval Service Delivery (FISD), Interval Delivery Conception (IDC), Pregnancy at First Service (PFS), Services by Concepción (SbC) and pregnancy rate (PR). Non-parametric variables were analyzed with a homogeneity test based on Chi2 ($p < 0.05$) and U test Mann-Whitney was used to compare independent continuous variables. From each cow a cytological sample of endometrial mucosa was tacked, using adapted endocervical brushes, and smears were air dried and fixed. Then taken to the laboratory to be colored by Diff-Quick staining. The cell reading fields where neutrophils, and used to determine the degree of inflammation of the uterine lining, obtaining a Percentage of polymorphs Nuclear Neutrophils (PMN-N%), associated to the total cells. The criteria for diagnosing subclinical endometritis positive (SE) was ≥5,10% of PMN-N in each smear (Reátegui, et al., 2015). In the Holstein breed cows positive IDC is 110.96 days to pregnancy, negative cows needed 98.47 days, the difference of 12 days between the two groups showed no statistically significant difference ($P = 0.070$ Mann Whitney U test) . In IPPS, positive cows showed 83.11 days as interval, negative cows showed 70.11 days, the observed difference is 13 days between groups, showing significant difference ($P = 0.019$). In the number of services per conception showed no statistically significant differences ($P = 0.241$) compared with healthy and SE cows, the positive needed 2,0 services to get pregnancy and the 1, 63 services for negatives. In Jersey breed no statistically significant differences were found when comparing positive and negative cows with subclinical endometritis for reproductive indices: IPC (96 vs 88.59 days, P=0.483), IPPS (62.77 vs 58.82 days, $P = 0.784$) and the number of services per conception SPC (2.31 vs 2.0, $P = 0.344$), respectively. We reported pregnancy rate (PR) of 42.86% for positive cows, and 42.86% for negative with ES in the Holstein breed. In the Jersey breed pregnancy rate (PR) is 38.89% with positive diagnosis 41.18 ES and negative diagnosis. There is statistically significant difference ($P < 0.05$) among genotypes for ES positive diagnosis. With this information, it is shown that a positive diagnosis subclinical endometritis negatively affects the reproductive efficiency of dairy cows. In addition, the genotype is a factor to take into account the distribution of subclinical endometritis and reproductive performance.

Keywords: Subclinical endometritis, polymorphonuclear, reproductive index

{12} THE EFFECT OF L-CARNITINE ON THE LEVEL OF INTRACELLULAR REACTIVE OXYGEN SPECIES, THE AMOUNT OF INTRACELLULAR LIPIDS AND THE NUCLEAR MATURATION OF IN VITRO PORCINE OOCYTES

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ABSTRACT

L-carnitine (LC) plays an important role in the catabolism of lipids and protects cells from the damage caused by reactive oxygen species (ROS) due to its antioxidant activity. The aim of this study was to evaluate the effect of the addition of different concentrations of LC during in vitro maturation (IVM) on the level of intracellular ROS, the amount of intracellular lipids and the percentage of nuclear maturation of porcine oocytes. The cumulus-oocyte complexes (COC) were obtained by follicular aspiration from ovaries of slaughtered sows and matured in vitro without LC (control) or with different concentrations (0,6, 1,25 or 2,5 mg/mL) of LC (Sigma-Aldrich) in the maturation medium (TCM-199, 10% porcine follicular fluid and antibiotics) for 44 h at 39°C and 5% CO2. Then, the oocytes were denuded and the level of intracellular ROS was evaluated with DCH-FDA test by the variable transmittance (T) of the fluorescent emission. The amount of intracellular lipids (Nile Red staining) was expressed as area red pixels/total area of the oocyte (R). The percentage of nuclear maturation was assessed by Hoechst 33342. Data were evaluated by a fluorescence microscope and analyzed by Statistix software. There were significant differences in the level of intracellular ROS between the control (T=97,24, n=104) and the concentrations of 0,6 and 1,25 mg/mL of LC (T=73,79, n=118; T=70,73, n=120, respectively). However, no significant differences were observed between the concentration of 2.5 mg/mL of LC (T=78,51, n=94) and the remaining groups (Kruskal-Wallis, p=0.01). The amount of intracellular lipids decreased significantly in the concentration of 0.6 mg/mL of LC compared to the control (R=729,11, n=111 and R=781,79, n=120, respectively), whereas, no significant differences were observed between the other groups (LC 1,25: R=750,18, n=120; LC 2,5: R=750,39, n=96) (Kruskal-Wallis, p=0,028). No significant differences were observed in the percentage of nuclear maturation between the control (66%, n=199) and the concentrations of 0,6 and 1,25 mg/mL of LC (59%, n=185; 62%, n=190 respectively), although, there was a significant decrease in the percentage of nuclear maturation between the control and the concentration of 2,5 mg/mL of LC (53%, n=192) (Fisher´s test, p=0,007). In conclusion, the addition of 0,6 mg/mL of LC in the IVM medium decreases the level of intracellular ROS and the amount of intracellular lipids of the oocyte compared to the control without affecting the percentage of nuclear maturation. Considering that the oxidative stress and the high concentration of lipid in porcine oocytes is detrimental to cryopreservation and embryo development, the use of 0,6 mg/mL of LC during IVM could improve the efficiency of these biotechnologies.

Keywords: L-carnitine, oocytes, porcine, ROS, nuclear maturation.

{13} USE OF INDUSTRIAL WIRELESS ENDOSCOPE IN INTRAUTERINE ARTIFICIAL INSEMINATION IN SHEEP

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ABSTRACT

Intrauterine artificial insemination (AI) in sheep allows use of low sperm count of high genetic value rams. However, the high cost represents laparoscopic equipment limits the applicability and dissemination of this AI technique. The aim of the study was to evaluate the pregnancy rate Hampshire down sheep inseminated into the uterus using a wireless industrial endoscope low-cost. The work was conducted between December 2015 and March 2016 in a farm Sullkataca Baja community in the Laja municipality, of La Paz Department, Bolivia, located at 3 960 masl. Endoscopy equipment (Wifi Endoscope®, Teslong) is a portable and multifunctional endoscope Wi-Fi camera, has a diameter of 8.5mm, with LED light and transmits real-time video to 720P HD video (AVI) and images (JPG) to mobile devices and was obtained in the local market at a price of $ 200,00 USD. Also, 2 metal trocars use in cattle were adapted to facilitate the entry of the camera and insemination device. Estrus synchronization 10 nulliparous and 10 pluripara ewes was performed by inserting intravaginal sponges with 50 mg of medroxyprogesterone acetate (Progespon®, Sintex) for 14 days and the withdrawal of the sponges a dose of 500 IU of eCG (Sergon®, Bioveta) was applied. A 52 hours after removal of sponges, AI at fixed time with frozen semen in 0,25 cc straws with $40x10^6$ sperm was performed. Handling sheep to inseminate was placing them on pivoting stretchers, proceeding to make two incisions in the abdominal region on skin and subcutaneous tissue, 4cm in front of the udder and 3cm in left and right lateral direction of the alba line to facilitate the entry of the trocars into the abdominal cavity through which the endoscope camera and aspic insemination were introduced respectively, depositing average dose in each uterine horn. The pregnancy diagnosis was performed 50 days post insemination using transrectal ultrasound (EMP 820 vet plus®, Emperor). Pregnancy rates were 60% for nulliparous and 70% for sheep. Statistical analysis (Chi square) for the rate of pregnancy among nulliparous and ewes did not show statistically significant difference between these two groups ($P \geq 0,05$). In conclusion, the use of Industrial wireless endoscope is economical, versatile and allows to obtain very good pregnancy rates in ewes inseminated in utero with frozen semen.

Keywords: ovine, artificial insemination, laparoscopy, wi-fi endoscope

{15} EVALUATION OF DNA INTEGRITY USING THE TECHNIQUE OF SPERM CHROMATIN DISPERSION IN CRYOPRESERVED EPIDIDYMAL SPERM

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ABSTRACT

The genetic integrity of the sperm is essential for the normal development of the embryo. A high level of DNA fragmentation in sperm cells may represent an important cause in male fertility. Reported studies have shown that regardless of reproductive biotechnology used, a high level of DNA fragmentation above the critical threshold, can significantly compromise the possibility of pregnancy. Sperm cryopreservation is a widely used in breeding centers, due to its great importance of preserving germplasm male animals of zoo technical value and those who may die unexpectedly biotechnical. It is art contains many processes in which cells or tissues are frozen at very low temperatures, usually between -80C and 196, which may reduce some physiological and biological activities that can affect post thaw sperm. Analysis of DNA fragmentation test as there TUNEL test, DBD-FISHER, among others, using extremely expensive reagents; however Sperm test Cromatin Dispersion (SCD) is much more practical and economical. The following study was to evaluate the rate of DNA fragmentation using the test of epididymal sperm SCD in post cryopreserved cattle. 15 epididymis of bulls from the slaughter of Lurin were used. Sperm were collected with the technique of retrograde flow using a diluent medium without cryoprotectant (Botubov®). They were analyzed the following parameters, motility, vigor, viability and integrity of DNA (fragmentation). The technique used for fragmentation Halosperm® kit consisted of a test based on the chromatin dispersion (SCD: Sperm Chromatin Dispersion) that is based on the differential response of fragmentation, or not, of the sperm nucleus to a treatment highlighting deproteinization fragments between the breakpoints. Extracted nuclear proteins of sperm with fragmented DNA by using a specific lysis solution to extract proteins, DNA loops constituting relax residual halos around the central core structure. For cryopreservation, sperm cells were separated into two samples and diluted in medium with cryoprotectant (Botubov®II) were packaged in 0.5 cc straws containing 50x106 motile sperm per straw. The samples were frozen by the conventional method (4 ° C for 4 hours in a refrigerator, and 20 minutes in a bowl of water). The percentages of fresh sperm motility were 63 and 26 % in frozen pos, compared to 3.0% effect was obtained in fresh versus frozen 1.8% after the viability versus 52 % 38% and DNA integrity (fragmentation) 98% was obtained in fresh sperm versus 91% after frozen sperm. We can see that with cryopreservation, significantly reducing all parameters of sperm quality evualuados, manage to maintain the viability of the sperm cells and can utlizarse asisitida reproduction programs. As for fragmentation is not a negative effect on sperm after freezing.

Keywords: Sperm, sperm fragmentation, semen, bovine.

{16} STALLION SEMEN: EFFECT OF TRANSFER TEMPERATURE AND EFFICACY OF ANDROCOLL-E™ AND GLASS WOOL FILTRATION PROCEDURES

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ABSTRACT

Reproductive management in the horse involves transport of semen supplemented with extenders and refrigerated to preserve sperm quality, followed by semen processing to select motile sperm, mainly by means of centrifugation through a colloid (Androcoll-E™). Another technique rarely used in the horse is the filtration through glass wool columns (GWF). This study aimed 1) To assess the effect of semen-transport temperature upon sperm motility and post-transport sperm selection efficacy by Androcoll-E™ selection procedure 2) To compare the efficacy of Androcoll-E™ and GWF to select sperm from fresh stallion semen. Twenty semen samples were collected from 4 stallions (3-12 yo) using an estrous mare and an artificial vagina (Missouri type). For 1), semen was supplemented with Kenney extender and transported for 1h at room temperature (RT, 22-25°C) or refrigerated (REF, 4-7° C). Then, both aliquots were processed with Androcoll-E™ (Minitube Int., Tiefenbach, Germany). For 2), diluted semen samples were transported at RT and processed in parallel by means of Androcoll-E™ and GWF (50 and 75 mg of GW; Manville-Fiber-Glass Corp; Denver, CO, USA) procedures. In 1) % of total (TM) and progressive (PM) sperm motility was determined. In 2), % of normal sperm form (NM), % sperm with osmotic-competent membranes (HOST+), and % of acrosome-intact sperm (AI, determined by FITC-PSA staining) only with osmotic-competent membranes (AI/HOST+) was also determined. The results were subjected to statistical analysis.1).The % of TM and PM sperm was similar in semen transported at RT or REF (%TM: RT= 65±9; REF=57±12; p=0.11; % PM, RT=32±17; REF= 26±8; mean±SDM; n=7; p=0.27; Wilcoxon test). Under both conditions, the % of post-Androcoll-E™ PM sperm was higher than in suspensions prior to selection (RT postA=45±15; REF postA=38±15; p=0.02 versus pre-selection; n=7; Wilcoxon test). 2) The use of GWF columns yielded similar results to those obtained with the Androcoll-E™ technique (n=13) for all sperm parameters evaluated:

Espermatozoides	Androcoll-E™	CLV-50 mg	CLV-75 mg	*p* (Test Friedman)
MT (%)	66±12	66±15	73±15	0,35
MP (%)	33±16	41±20	43±22	0,63
MN (%)	61±13	61±11	64±12	0,26
HOST+ (%)	48±9	57±10	57±9	0,07
AI/HOST+ (%)	73±26	71±17	68±16	0,96

En conclusion. 1) Stallion semen diluted in Kenney extender and transported for 1h at RT preserves the same sperm motility and ability to be selected by Androcoll-E™ as that of semen transported in refrigeration, 2) GWF is an acceptable alternative procedure to centrifugation through Androcoll-E™ for selection of motile, morphologically-normal, membrane-competent and acrosome-intact sperm from stallion fresh semen.

Kerwords. Semen, stallion, centrifugation, filtration.

{17} CULTURE AND PURIFICATION OF PORCINE CORPUS LUTEUM CELLS AS A SUBSTRATE FOR IN VITRO MATURATION OF PORCINE CUMULUS OOCYTE COMPLEXES. ESTABLISHMENT AND CHARACTERIZATION

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ABSTRACT

Embryos in vitro production is still an inefficient biotechnology compare with the results obtained by oocytes matured and fertilized in vivo. Nuclear and cytoplasmic maturation has not been well described in porcine as it has been done in other species, reflecting poor pronucleus formation and high incidence of polyspermic fertilization. The establishment and maintenance of a suitable in vitro microenvironment plays an essential role in maturation and subsequent fertilization, whereby the utilization of a standardized coculture would recreate in a better way the in vivo microenvironment. The choice of porcine corpus luteum (CLP) cells in monolayer for coculture with cumulus oocyte complexes (COC) is based on the steroidogenic function of these cells with basal progesterone (P4) production. This hormone has an antiapoptotic effect due to a down regulation of Fas expression and its antioxidant effect. At the same time, P4 is a mediator in the meiotic resumption induced by an increase of gonadotropins. The objective of this study was to establish and characterize the CLP culture cells for the subsequent coculture with porcine COC. The final purpose is to decrease the levels of oxidative stress and apoptosis induced by reactive oxygen species (ROS), promoting oocyte maturation. CLP culture was established and purified using corpora lutea obtained from slaughterhouse ovaries. Corpora lutea were dissected and luteal tissue submitted to a mechanical and enzymatic digestion with collagenase IV. The cell suspension was filtered and centrifuged and the cells obtained were diluted in 10 mL of DMEM-F12 supplemented media. Diluted cells were seeded in 2 culture flasks T25, staying in a controlled environment and changing the medium every 2 days. Passage 1 cells were submitted to a Percoll® density gradient centrifugation. For the analysis and characterization, the cells were assessed by intracellular lipid content using the Red Nile staining, immunocytochemistry (ICC) for 3β-hydroxy steroid dehidrogenase (3β-HSD) and ELISA for P4 determination. We observed the presence of lipid intracellular granules with differential characteristics in the different phases recovered from the Percoll® density gradient centrifugation. Also, we observed crescent measurements of P4 at 48, 96 y 144 h of primary culture and almost all the cells were positive to the ICC evaluation for 3β-HSD, showing the steroidogenic capacity of the culture cells. Preliminary assays were done for in vitro maturation of COC in coculture with CLP. Nuclear maturation was evaluated observing, until this moment, similar percentages between the treatment and the control (with hormones). These results would allow us to optimize in vitro embryo production and its possible technological transference to the productive systems.

Keywords: Coculture, corpus Luteum, in vitro maduration, porcine.

{18} INTRAUTERINE APPLICATION OF FLAVONOIDS DURING BOVINE PUERPERIUM AND ITS EFFECT ON REPRODUCTIVE TRACT INVOLUTION AND FERTILITY IN DAIRY COWS

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ABSTRACT

During postpartum period, known as puerperium, bovine species requires time for reproductive tract involution and for resumption of oestrous cycles. Several studies have demonstrated the potential effects of flavonoids as anti-bacterial and anti-inflammatory agents. The main objective of the present study was to evaluate the effects of intrauterine administration of an active ingredient based on natural flavonoids in order to hasten reproductive tract involution and to shorten the parturition-to-conception interval in dairy cows. A total of 40 Holstein cows (2^{nd}-3^{rd} lactation; BC: 3-3.5) were enrolled in the present study. The cows were divided randomly into 4 experimental groups [1 control group (T1) and 3 treatment groups (T2, T3 and T4)]. Treatments consisted of one single intrauterine administration on day 10 postpartum of 90 mg (T2), 180 mg (T3) and 360 mg (T4) of ultrapure flavonoid powder dissolved in destilled water excipient to make 20 ml of solution. Ultrasonographic measures (Aloka SSD-500, 5 MHz, Japan) of different reproductive tract anatomical structures (cervix, uterus and ovaries) were scored 3 times during the postpartum period (day 10, 15 and 21 postpartum). Scores taken from cervix were: length, width and thickness; from uterus: diameter and thickness; and finally, from ovaries (left and right): length and width. As fertility parameters rate of return to estrus [(Total N° of cows in estrus / Total N° cows) x 100], pregnancy rate [(Total N° of pregnant cows / Total N° inseminated cows) x 100] and calving-to-conception interval were scored as well. Pregnancy status was performed by using ultrasonography (day 35 post-insemination). ANOVA was carried out after a preliminary examination of the data (SPSS software v.15 for Windows). Subsequently, multiple comparison analysis post-hoc Scheffe test was performed after the determination of differences among means. For percentage analysis of variables, chi-square test was applied. The differences were considered statistically significant at $p < 0.05$. There were multiple differences ($p < 0.05$) observed among reproductive tract structure dimensions (cervix and uterus) in values obtained from T1, T2, T3 and T4 groups after ultrasonographic analysis at day 10, 15 and 21. No significant differences were observed ($p > 0.05$) in ovarian dimensions (right and left ovaries) from T1, T2, T3 and T4 groups during the postpartum period after ultrasonographic analysis at day 10, 15 and 21. Return to estrus index, pregnancy rate and calving-to-conception interval differed between T1 and T2-T3-T4 groups ($p < 0.05$), however, no differences were observed among T2, T3 and T4 groups ($p > 0.05$). In conclusion, intrauterine application of 90 mg (T2), 180 mg (T2) and 360 mg (T3) flavonoid solution had a strong tendency to increase cervical and uterine involution. Finally, although no significant differences among treatments, the intrauterine application of flavonoid solution improved return to estrus index, pregnancy rate and calving-to-conception interval compared with non-treated dairy cows, being T3 the election treatment in relation to cost-benefit analysis.

Keywords: Puerperium; Flavonoids; reproductive tract; involution; fertility

{19} DEVELOPMENT OF A TECHNIQUE FOR SPERM CAPACITATION ASSESSMENT USING FLOW CYTOMETRY IN ALPACA SPERMARTOZOA

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ABSTRACT

Usually, sperm capacitation have been evaluated by microscopy fluorescence using chlortetracycline (CTC). In this technique it is possible to identify three patterns of spermatozoa: uncapacitated (F), capacitated (B) and acrosome reacted (AR). However, flow cytometry evaluation has not been able to distinguish these three populations; because CTC fluorescence intensity of B and AR patterns are similar. Therefore, the objective of this study was to develop a method to evaluate sperm capacitation in alpacas by flow cytometry. Testicular/epididymis of alpaca were obtained from the Municipal Ninacaca Slaughterhouse, Pasco. Sperm retrieval was performed using serial cuts from the epididymis tail, and cells were suspended in PBS. In order to establish the positive controls for each population of interest, we worked with raw spermatozoa (lower proportion of capacitation), cryopreserved spermatozoa (increased proportion of capacitation), capacitation induced spermatozoa (incubated with BWW capacitating medium at 37.5°C) and induced acrosome-reacted spermatozoa (incubated calcium ionophore 10 μM). All samples were incubated at 37.5°C with 750 μM of CTC for 20 minutes, then FITC-PSA (2.5 mg / mL) and propidium iodide (PI: 5 μg / mL) were added and incubated for additional 10 minutes at 37.5°C. Evaluation by flow cytometry was performed using a FlowSight (Amnis) cytometer with 488 nm and 405 nm excitation lasers; and using channel 8 (Ch-08: 505-560 nm) for CTC, channel 2 (Ch-02: 505-560 nm) for FITC-PSA and Channel 5 (Ch-05: 642-740 nm) for PI. By flow cytometry assessment the population of viable spermatozoon (PI negative) was selected, and this population was assessed by a dot plot of Ch-02 and Ch-08 to assess simultaneously the fluorescence intensity of FITC-PSA and CTC. Uncapacitated spermatozoa (F) were CTC-positive and FITC-PSA-negative; capacitated spermatozoa (B) were CTC-negative and FITC-PSA-negative; while acrosome-reacted spermatozoa (AR) were CTC-negative and FITC-PSA-positive. In this way it was possible to differentiate B from AR spermatozoa (both CTC-negative) by adding FITC-PSA, because capacitated spermatozoa (B) were FITC-PSA was negative, meanwhile acrosome-reacted spermatozoa (AR) were FITC-PSA positive. Our results showed sperm populations grouped as expected; where raw sample showed a larger population in region CTC-positive and FITC-PSA-negative (F); cryopreserved spermatozoa showed a heterogeneous distribution predominantly in region CTC-negative and FITC-PSA-negative (B); capacitation induced spermatozoa showed a larger population in region CTC-negative and FITC-PSA-negative (B) and induced acrosome-reacted spermatozoa showed a larger population in region CTC-negative and FITC-PSA-positive (AR) . In conclusion, it is possible to evaluate sperm capacitation in alpaca spermatozoa by flow cytometry using simultaneously CTC, FITC-PSA and PI fluorochromes.

Keywords. Sperm capacitation, flow cytometry, alpaca.

{20} CORRELATION BETWEEN MITOCHONDRIAL MEMBRANE POTENTIAL AND TOTAL MOTILITY IN ALPACA SPERM

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ABSTRACT

The mitochondrial membrane potential (MMP) is a parameter that could be highly related to the functionality of alpaca spermatozoa. Any failure in MMP could directly affect sperm motility and therefore fertilization capacity. The objective of the study was to determine the correlation between mitochondrial membrane potential and total sperm motility in alpaca spermatozoa. Thirty-one alpaca testicles were obtained at the Municipal Slaughterhouse in Ninacaca, Pasco. They were transported in NaCl (0.9 %) at 5 °C for about 20 hours. Spermatozoa were retrieved from the tail of the epididymis through serial cuts, sectioning and pressing them for release. Spermatozoa were recovered in 1 mL of TRIS-base extender (Tris 2.71g, 1.4g citric acid, 1 g fructose) at 37 ° C. Total sperm motility of each sample was evaluated subjectively, placing 10 μL in microscope slides and observed under an optical microscope at 400X. To assess MMP, MitoTracker Red 633 Deep FM (Invitrogen Molecular Probes, M24426) and MitoTracker Red CMXRos (Invitrogen Molecular Probes, M7512) were used. Each sample were aliquoted into 2 parts of 100 μL. One aliquot was incubated with 0.5 μL of MitoTracker Deep Red 633 FM (20 μM) to reach a final concentration of 100 nM; while the other aliquot was incubated with 0.5 μL of MitoTracker CMXRos (20 μM) to reach a final concentration of 100 nM. Aliquots were incubated for 10 minutes at 38 ° C in the dark for further evaluation. Samples were evaluated using a flow cytometer FlowSight (Amnis) being acquired 10,000 events for each one. The events were excited with a 642 nm wavelength laser for MitoTracker Red 633 Deep FM and 488 nm wavelength laser for MitoTracker CMXRos. Fluorescence emission was read in channel 11 (Ch11: 642-740 nm) and channel 4 (Ch4: 595-642 nm), respectively. Spermatozoa with high florescence in the mid piece were considered as spermatozoa with high MMP, where we observed red fluorescence for MitoTracker 633 Deep Red and orange fluorescence for MitoTracker CMXRos. Results of sperm with high MMP were expressed as percentages in a histogram graph. Total motilities between 20 to 80 % were obtained, with an average of 37.58 ± 13.47. The 59.52 ± 19.19 % of spermatozoa showed a high MMP for MitoTracker Red 633 Deep Red 633 FM and 65.03 ± 15.92 % for MitoTracker CMXRos. Test Pearson correlation (r) was performed to compare the MMP with total motility. Comparisons between total motility and high MMP for MitoTracker CMXRos showed a significant moderate correlation (r = 0.39, p 0.05), while for total motility and high MMP for MitoTracker Deep Red 633 was not significant (r = 0.02, p = 0.91). It is concluded that there is a moderate correlation between sperm motility and MMP determined by MitoTracker CMXRos in alpaca spermatozoa.

Keywords. Mitochondrial membrane potential, motility, sperm, alpaca.

{R-22} EFFECT OF MATING TIME OVER REPRODUCTIVE AND PRODUCTIVE PARAMETERS IN GUINEA PIGS (Cavia porcellus), AT RAIN FOREST.

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ABSTRACT

The limited use of reproductive biotechnologies in guinea pigs and try to maximize the male use in this species led to perform this work in order to determine the effect of time of mating and subsequent matings on reproductive and productive parameters in guinea pigs breeding in humid tropics, at an average annual temperature of 25°C, an average annual rainfall of 3200 mm to 660 meters high. The work was done in the guinea pigs production barn of Animal Science Farm of the National University Agrarian of the Forest, Tingo Maria - Peru, using 78 guinea pigs: 72 females and 6 males, 36 females of Andean race and 36 of Perú race; females were three months old weighing approximately 750 g, males were brought from another farm to avoid inbreeding problems, from same race (Peru), similar age (four months) and weight (1 kg approximately). A completely randomized block design with factorial arrangement was used, being the factors evaluated: mating time (14D and 21D days), mating turn or mating number (1ST and 2ND) and males, being the Female race the blocked variable. Reproductive parameters evaluated were: birth rate (TP), total litter size or prolificacy (TCT) viable litter size or viability (TCV); and reproductive parameters evaluated were: average weight at birth (BW), average weaning weight (PD) and mortality rate at birth (MN) and at weaning (MD). As a result, it was showed statistically significant differences between mothers weights at beginning of breeding season, childbirth and postpartum, being higher at birth, also was observed that the interval between the first and last childbirth within each mating group (cage) ranged from 5.60 to 6.71 days. Reproductive parameters evaluated show that TP presented statistically significant differences in mating time ($P = 0.029$), the male used ($P = 0.0052$) and the interaction between mating time and mating turn decreasing the birth rate from the 1ST to 2ND mating turn in the mating time of 14D (from 94.45 to 83.33%) for the TCT there were statistical differences in mating time ($P = 0.0242$) factor and the interaction between the factors mating time and mating turn, for TCV were only statistically significant differences in the interaction between the factors mating time and mating turn following the same trend as the TCT. Respect production parameters, there were only statistically significant differences ($P <0.05$) for the PN and PD in mating time, male and female race factors, and there were none in MN or MD, for any of the factors. In conclusion, the interaction between mating time (14D or 21D) and mating turn (1st and 2nd), only affect reproductive parameters (PT, TCT, TCV), while there is any effect on the production parameters of guinea pigs raised in the humid tropics.

Keywords: Guinea Pigs, mating time, mating turn, breeding animal.

{23} RELATIONSHIP BETWEEN MOTILITY AND VIABILITY/ACROSOME INTEGRITY IN ALPACA EPIDIDYMAL SPERMATOZOA

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ABSTRACT

The evaluation of sperm motility is usually evaluated subjectively and is considered related with male fertility ability. Furthermore, assessment of percentage of acrosome-intact viable spermatozoa is a parameter that requires a more complex processing methodology, nevertheless, it is considered an indicator of optimum quality in semen samples. Therefore, the objective of this study was to determine the relationship between the percentage of sperm motility and percentage of acrosome-intact viable in epididymal spermatozoa in alpacas. We worked with fourty-five testicles obtained from Ninacaca´s Municipal Slaughterhouse, in Pasco. Testicles were placed in plastic bags with solution of sodium chloride (NaCl) 0,9% and were transported at 5 °C to Lima. In Lima, cauda epididymis were separated and epididymal sperm samples recovered. Sperm progressive motility was evaluated in all samples with microscopy at 400 X. Then, samples were washed by centrifugation with PBS and re-suspended in two aliquots of 100 µL. One aliquot was incubated with FITC-PSA (2,5 µg/mL) and propidium iodide (5 µg/mL); while the other aliquot was incubated with FITC-PNA (0,5 µg/mL) and propidium iodide (5 µg/mL). Both aliquots were incubated for 8 minutes at 38° C. Percentage of acrosome-intact viable spermatozoa was evaluated using a flow cytometer FlowSight (Amnis) equipment. Pearson correlation was used to evaluate the correlation between motility and AIV spermatozoa obtained by FITC-PSA and by FITC-PNA. Additionally, samples were divided into three groups according to their initial motility: samples with low motility (under 30%) regular motility (between 30% and 40%) and good motility (Over 40%). Motility was 13,41 ± 6,26 (Low group), 35,00 ± 4,18 (regular group) and 52,50 ± 8,86 (good group). Correlation coefficients between motility vs. acrosome-intact viable spermatozoa for FITC-PSA and FITC-PNA were 0,33 and 0,31 respectively. In addition, the coefficients of correlation between sperm motility and acrosome-intact viable in low, regular and good motility groups were 0,54, 0,32 and 0,64 for FITC-PSA; while they were 0,45, 0,44 and 0,45 for FITC-PNA, respectively. These results indicate that there is an average positive correlation between the evaluated variables, however, this correlation is higher when working with samples with greater than 40% motility and sperm assessment is performed using FITC-PSA. It is concluded that the assessment of sperm motility may be a moderate indicator of the viability and acrosome integrity in alpaca spermatozoa with initial motilities over 40%.

Keywords. alpaca, spermatozoa, motility, acrosome, flow cytometer

{24} EVALUATION OF VIABILITY AND MITOCHONDRIAL MEMBRANE POTENTIAL IN ALPACA SPERMATOZOA CRYOPRESERVED IN DIFFERENT EQUILIBRATION PERIODS

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ABSTRACT

Sperm viability and mitochondrial membrane potential are two parameters related with spermatozoa fertilizing ability. During the equilibration period in cryopreservation, alpaca spermatozoa undergo oxidative stress, and it is possible that their fertilizing ability could be altered negatively. The objective of this study was to determine differences between percentages of sperm viability and mitochondrial membrane potential in alpaca post-thawed spermatozoa cryopreserved using four different equilibration periods in the freezing protocol. Twenty-four alpaca testicles were obtained from Municipal Slaughterhouse in Ninacaca, in the department of Pasco, transported at 5°C in a ClNa (0,9%) solution for 20 hours. Cauda epididymis was separated from the testicles and put in a Petri dish, 1 ml of milk and egg yolk extender was added and serial sections were made to obtain spermatozoa. Each sample (n=24) was loaded into plastic straws to proceed with the freezing process using program number 7 in an automatic freezing device (Freeze Control Cryologyc®). Once 5°C were reached, samples were maintained at this temperature for 0 (T1), 5 (T2), 15 (T3) and 30 (T4) minutes. After this, samples were exposed to liquid nitrogen vapor at a distance of 10 cm for 15 minutes and stored in a liquid nitrogen tank. Samples were thawed at 37°C for 1 minute, and then each sample was washed twice by centrifugation with PBS solution. Pellets were reconstituted in 100 µl of PBS and 0,5 µl of MitoTracker Deep Red 633 FM (20 µM), 0,5 µl of SYBR-14 (20 µM) and 0,5 µl of Propidium Iodide (5 µg/ml) were added and then incubated for 10 minutes at 38°C. Finally samples were evaluated using a flow cytometer FlowSight (Amnis). Ten thousand events were acquired for each sample, using a 642 nm wavelength laser for MitoTracker Deep Red 633 FM and 488 nm wavelength laser for SYBR-14 and Propidium Iodide. Spermatozoa with a green fluorescence on their heads were considered viable and the ones with an orange fluorescence in the mid piece were considered as spermatozoa with high mitochondrial membrane potential. Spermatozoa that showed a red fluorescence on their heads were considered as non-viable spermatozoa. Treatment averages were analyzed using ANOVA test. Percentages of viable spermatozoa (T1: 17,2 ± 8,05%, T2: 15,4 ± 9,7%, T3: 19,31 ± 12,33% y T4: 17,85 ± 7,65%, mean ± SD) and with high mitochondrial membrane potential (T1: 21,66 ± 9,27%, T2: 16,85 ± 8,44%, T3: 18,69 ± 7,32% y T4: 20,30 ± 8,41%, mean ± SD) were similar in all groups (P>0,05). We can conclude that working with spermatozoa recovered form cauda epididymis, under any of the equilibration periods, would not generate significant changes over sperm viability and mitochondrial membrane potential.

Keywords. Mitochondrial membrane potential, viability, spermatozoa, cryopreservation, alpaca

{R-25} LLAMA SPERM MORPHOLOGY (*Lama glama*) IN UNSTAINED CELLS USING THE TRUMORPH ®

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ABSTRACT

Sperm morphology is essential in the analysis of semen of animals. Trumorph ® is a device developed to prepare sperm samples to assess their morphology without the need for staining by a negative contrast phase microscope. The objective of the study is to test if this technique can assess llama sperm morphology and determine whether there effect of the animals studied in sperm shapes. The study conducted between March and April 2016, at the Centro de Investigaciones en Camélidos Sudamericanos (CICAS) La Raya. The collection by vaginal aspiration was performed on 3 males llamas between 4 and 5 years suitable reproductively, the semen collection was performed on 3 occasions with one week intervals using a proctoscope and 15 ml collection tube. Sperm samples were prepared with Trumorph® (Proiser R + D, SL, Paterna, Spain), for which 5 µl sample was deposited on a microscope slide, a cover slip was placed and introduced in the Trumorph®, where the sample was under a pressure of 6 kp and 60 ° C for 6 seconds. The sperm morphology was evaluated with Integrated Semen Analysis System - ISAS® (Proiser R + D, Paterna, Spain) with a negative phase contrast 40X objective, 200 sperm per sample were evaluated and classified into normal sperm, pyriform, elongated, thin and rounded (Soler *et al.,* 2014). The data did not show normal distribution (Kolmogorov-Smirnov test), or variances homogeneity (Levene test), so the Kruskal-Wallis test was used to determine whether the effect of the animals in the form of sperm, all these analyzes were performed with SAS. Was obtained 54,72 ± 6,57% of normal shape sperm, the pyriform were 10,94 ± 4,20%, 22,44 ± 4,46% elongated, thin 8,06 ± 3,33% and rounded sperm were 3,84 ± 1,95%. No significant differences (p> 0,05) between animals for sperm shape, similar to that reported by Soler *et al.,* 2014. It was found that the Trumorph® can identify the shape of the llama sperm without dye them. It has found 5 head sperm shapes: normal, elongated, thin, pyriform and rounded, no significant were found in the shape sperm between animals.

Keywords. Trumorph®, sperm, morphology, llama

{R-26} OSMOTIC TOLERANCE AND ACROSOMAL INTEGRITY IN SPERM ALPACA RECOVERED FROM THE VAS DEFERENS

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ABSTRACT

Sperm recovered from the vas deferens are an alternative for work in vitro, that is why arises evaluate viability, functionality and acrosomal membrane integrity of sperm recovered from the alpaca vas deferens in media with different osmolarity and carbohydrates. The study was conducted between November 2015 and February 2016, at the Centro de Investigaciones en Camélidos Sudamericanos (CICAS) La Raya, Cusco, Perú. Sperm retrieval was performed with 0,3 ml of Tris (300 mOsm/L) in 3 adult male alpaca with surgical diversion duct and held in grazing. Four collections weekly intervals per animal was conducted. Each recovered sample was divided into 4 aliquots of 50 µl to which was added 500 µl of diluters which 250, 300, 350mOsm/L with fructose and 300 mOsm/L with glucose. The osmolarity verified with electronic osmometer. Evaluations were performed after incubation keep them for 5 minutes at 37 ° C. The vitality was determined with VitalTest® in fluorescence microscope and functionality membrane evaluated with the hypoosmotic test in 50 mOsm/L solution (1: 4) incubating for 5 minutes and evaluated at 400X. Acrosome integrity was determined by Coomassie blue staining (Giuliano et al., 2012) evaluated immersion oil in field clear to 1000X. The variables were analyzed randomized block design with Tukey test (parametric) and Kruskal-Wallis with C-Dunnet (nonparametric) using SPSS. No significant differences ($p>0,05$) were found between four different dilutors for vitality, functionality and acrosomal membrane integrity, it is on average vitality 36,8 ± 12,0%, membrane functionality 34,6 ± 11,5% and acrosome integrity of 36,6 ± 19,2%. These results demonstrate that the vas deferens alpaca sperm have a tolerance or adaptive capacity to dilutors in a range of 250 to 350mOsm/L, without altering sperm viability or functionality of the membrane. However, the high percentage of sperm with damaged acrosome could reduce the efficiency of in vitro procedures.

Keywords. Alpaca, sperm, viability, membrane functionality, acrosome.

{R-27} MORPHOLOGICAL ANALYSIS OF ALPACA SPERMATOZOA (*Vicugna pacos*) IN UNSTAINED CELLS USING THE TRUMORPH ®

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ABSTRACT

Sperm morphology is essential in the analysis of semen of animals. Trumorph ® is a device developed to prepare sperm samples to assess their morphology without the need for staining by a negative contrast phase microscope. The objective of the study is to test if this technique can assess sperm morphology alpaca recovered from the vas deferens and collected by vaginal aspiration. The study was conducted between January and April 2016, at the Centro de Investigaciones en Camélidos Sudamericanos (CICAS) La Raya. 6 males aged between 4 and 7 years old were used, 3 for the collection of semen by vaginal aspiration, and 3 with the vas deferens surgically diverted to the inner thigh. For the recovery of spermatozoa from vas deferens was used 0,3 ml of Tris base in 2 ml vials tubes, it was performed twice a week; the collection by vaginal aspiration was performed once a week, collecting the seminal flow of the external os of the cervix, both methods were obtained 3 samples per animal. Sperm samples were prepared with Trumorph®, for which 5 µl sample was deposited on a microscope slide, a cover slip was placed and introduced in the Trumorph® (Proiser R + D, SL, Paterna, Spain), where the sample was under a pressure of 6 kp and 60 ° C for 6 seconds. The sperm morphology was evaluated with Integrated Semen Analysis System - ISAS® (Proiser R + D, Paterna, Spain) with a negative phase contrast 40X objective, 200 sperm per sample were evaluated, it were classified into normal spermatozoa, pyriform, elongated, short and rounded (Buendía *et al.,* 2002); t test was performed with the percentages of sperm collected by the 2 methods, the SAS was used for analysis. Retrieved sperm in the vas deferens, normal were 54,99 ± 9,31%, 14,16 ± 3,30% pyriform, elongated 21,32 ± 5,75%, 6,05 ± 3,29% short and rounded 3,48 ± 2,56. In sperm collected by vaginal aspiration 52,79 ± 8,55% were normal, pyriform 12,31 ± 5,71%, 25,54 ± 6,50% elongated, 7,23 ± 2,47% short, and 2,13 ± 2,27% sperm rounded. No significant differences (p>0,05) were found in all forms sperm collected by the 2 methods. The percentages of normal, rounded and elongated sperm are similar to those reported by Buendía *et al.*, 2002, not the pyriform and short. It was found that the Trumorph® can identify the shape of the alpaca sperms without dye them. We found 5 shapes sperm head: normal, elongated, short, pyriform and rounded, finding no statistical differences in the vas deferens recovered and collected by vaginal aspiration.

Keywords. Trumorph®, sperm, morphology, alpaca

{28} THE EFFECT OF SEMINAL PLASMA CONCENTRATIONS ON THE OXIDATIVE DAMAGE IN EPIDIDIMAL THAWED ALPACA SPERMATOZOA. PRELIMINARY ASSAY

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ABSTRACT

It has been described that viscosity of seminal plasma in alpacas limit contact between the sperm cell membrane and cryprotective compounds during cryopreservation. However, it has been considered that the complete dilution was detrimental to sperm function, because it has antioxidant compounds that helped to avoid the oxidative damage during the process. That is why the objective of this assay was determining the effect of seminal plasma concentrations on the functional characteristics of the epididymal thawed alpaca spermatozoa during the cryopreservation process. Samples of semen collected from 3 male alpacas with proven fertility, were centrifuged to obtain seminal plasma (PS) and then stored in nitrogen liquid. The spermatozoon were recovered from 10 epididymal tails, and transported at 5°C during 20 hours from a slaughterhouse in Ninacaca – Cerro de Pasco. The sperm was suspended in 1ml of extender based on UHT skim milk (19ml), egg-yolk (1ml), D-fructose (0,960g) and Dimethylacetamide (1840µl). It was divided in 4 groups in which each one were added seminal plasma in different concentrations 0, 5, 10 and 15%. Then they were put in plastic straws and frozen, using a controlled freezing chamber CL5500 from Criologic® using programme N°7, and storing in a liquid nitrogen tank at -196°C. Once they were thawed in water bath at 37,5°C for 60 seconds, were washed by centrifugation (600 RFC for 8 minutes) and the pellet was resuspended in PBS. It was took 10µl to read progressive motility post thaw and each sample was incubated with 100nM of SYBR14, 5 µg/mL of Propide Iodide (PI) and 100 nM of MitoTraker Deep Red 633 for 10 minutes. Measurements were made using an FlowSight (Amnis) cytometer, with excitation lasers from 488nm to 642nm; and evaluating the fluorescence emission of SYBR 14 in channel 2 (Ch02: 505 to 560 nm), PI in channel 5 (Ch05 642 to 740 nm) and MitoTracker Deep Red in channel 11 (Ch: 642 to 740 nm). Percentage of viable spermatozoa were determined (SYBR14 positive; PI negative) and spermatozoa with high mitochondria membrane potential (MitoTracker Deep Red positive). Percentages of motility post thawed were 23,20 ± 9,18 (0% PS), 10,2 ± 7,49 (5% PS), 13,4 ± 6.81 (10% PS) and 14,8 ± 6,57 (15% PS), not existing difference between groups ($p > 0.05$). In the same way, percentage of viability (15,12 ± 5,25, 20,49 ± 5,01, 16,58 ± 1,96 and 17,70 ± 1,51% for 0, 5 10 and 15% of seminal plasma respectively) and spermatozoa with high mitochondria membrane potential (16,80 ± 4,84, 23,10 ± 4,71, 19,42 ± 2,01 6 and 18,94 ± 1,05%, for 0, 5, 10 and 15% of seminal plasma respectively) were similar between all groups ($p > 0,05$). It is concluded that freezing alpaca sperm with seminal plasma (0-15%) does not affect sperm functionality post thawed, however it is still necessary to develop more assays.

Kerwords. Seminal plasma, sperm, cryopreservation, alpaca

{29} EFFECT OF ENERGY SUPPLEMENTATION ON REPRODUCTIVE EFFICIENCY IN ALPACAS

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ABSTRACT

In a changing world as we face today, alpacas can not be excluded from the increasingly important "globalization" and competitiveness. Low fertility is one of the critical problems in the reproductive performance of the high Andean camelids from Peru and other countries areas; therefore, their production yields are affected, partly because of reproductive problems. Under the problematic one trial in order to determine the effect of energy supplementation on reproductive performance was carried out, using indicators such as ovarian activity (14d postpartum), service charge (21d), pregnancy rate (60d) and birth rate in alpacas. The study was conducted at the farm Pacomarca Inca SA Group, to 4060m above sea level in the province of Melgar-Puno, with air temperature between -17 ° and 21 ° C., Using 60 pregnant female alpacas 1st, 2nd and 3rd delivery, divided into two groups (with and without additional cost). The supplementation period was for 25 days and after childbirth, with a mixture of ground corn and mineral vitamin supplement in fresh cow's milk in an amount of 100 g of dry matter per day (PC 13.8% and EM 3.68 Kcal / g MS), and its own technical management of the farm, with adjustments to management alpacas Australia, United States and New Zealand, consisting feed on natural pastures of Fedo-Mufa good condition association, for 8 hours (9-17 h), forage supplementation hay and silage oats in the morning (7-8 h), especially in the dry season (June to September). The diagnosis of ovarian activity, service and pregnancy rate was determined by transrectal ultrasound; body condition, inspection and palpation. Data were evaluated by analysis of variance in Full Design Random main effects model GLM (General, Model Line). The service rate was analyzed by Chi square test through statistical program (α = 0.05). The results indicate that supplementation had no effect on reproductive variables. Alpacas supplemented showed higher number (1.52 vs. 1.31) (P = 0.0021) and follicles size (8.68mm vs. 9.14) (P = 0.0123) of the unsupplemented (P < 0.05); higher rates of service (80 vs. 53.3%) (P = 0.2845), gestation (93.3 vs. 73.3) (P = 0.3523), birth (66.7 vs. 53.3%) (P = 0.4216) and body condition score (3.8 vs. 3.4) (P = 0.0017), the unsupplemented, respectively. From the results, it is concluded that energy supplementation has a positive effect on reproductive performance in alpacas.

Keywords: body condition, reproductive performance, fertility, supplementation

{30} EFFECTS OF COLLECTION DAY ON EMBRYO RECOVERY AND PREGNANCY RATES IN EMBRYO TRANSFER IN ALPACAS

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ABSTRACT

In camelids, embryo transfer is performed between 7 and 8 post breeding season, regarded as optimal recovery rate ≥7 day post mating, as recovery rates of embryos before this time (day 6) are low; while those performed at day 8 appear higher. Thus the present study was conducted aimed at evaluating the effect of embryo collection day without the application of superovulation treatment, recovery rate of embryos and pregnancy, Center breeding Pacomarca located in Llalli-Melgar-Puno (29°21'01 "south latitude, 82°41'57" west longitude), at 4060m altitude. 21 Huacaya alpacas were used as donors, collected every 10 days for a period of approximately 70 days, achieving an average of 7 per animal collections during this period. The criterion for inclusion in donor was the genetic value, reproductively females with more than two births and clinically healthy. 138 alpacas as recipients (90 of huacaya and 48 of Suri breed), whose inclusion criterion was good mothering ability; greater than 5 parts were used. Synchronization donor, was performed by intramuscular administration of a dose of buserelin acetate 4,2µg (day -10) and 0,25 mg of sodium cloprostenol (day -1) and mounted with male fertility checked at day 0. Embryo collection was performed on 8 and 8,5 post breeding. Receiving synchronization was performed with the same protocol, except that the day + 1, a 4,2µg buserelin dose was applied. The presence of Corpus luteum was checked by ultrasonography (5 MHz) rectally before application of sodium cloprostenol, apply the product only to females presence of Corpus luteum. The pregnancy rate was evaluated through a Chi-square test; while the results of the recovery rate of embryos once converted to angular values were subjected to analysis of variance, the significance level used was ($p \leq 0,05$), analyzed using the SAS statistical program. The recovery rate of embryos was significantly higher ($p < 0,05$) when the uterine lavage day 8,5 (94,12%, 48/51) than when the 8 (81,81%, 90 was made as / 110). Pregnancy rate was similar ($p > 0.05$) for embryos recovered at 8 days (64.58%, 31/48) and 8.5 days (65.52%, 19/29) in the Huacaya breed and embryos recovered at 8 days (73,91%, 17/23) and 8,5 days (81,25%, 13/16) in the suri breed. In conclusion, the best time for uterine lavage in alpacas is 8,5 days, and then performs embryo transfer either between 8 and 8,5, because the day of transfer has no effect on the pregnancy rate.

Keywords: embryo transfer, huacaya, suri, pregnancy, alpacas.

{R-32} FIRST LLAMA BORN BY IN VITRO FERTILIZATION OBTAINED OF GAMETES DERIVED ABATTOIR

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ABSTRACT

The use of in vitro fertilization (IVF) in camelids could be an alternative for the genetic improvement of domestic camelids and for the preservation of wild species. The aims of this case study were to transfer alpaca and llama embryos obtained by in vitro fertilization into recipient llamas and evaluate pregnancy and birth rates. Were used gametes obtained from ovaries and testes of animals slaughtered at the abattoir of Huancavelica. Cumulus oocyte complexes (COCs) were recovered by aspiration of ovarian follicles using a 5 ml syringe, they were matured in vitro for 30 and 36 hours for alpaca and llama respectively. Then, COCs matured were transferred to in vitro fertilization FERT-TALP medium and inseminated with sperm recovered from the cauda epididymis (3×10^6 sperm/ml) which had between 70 - 80% motility. For the recovery spermatozoa SPERM-TALP medium was used and for the selection of motile spermatozoa swin up technique was used. Before in vitro insemination, spermatozoa were capacited for 30 minutes in FERT-TALP medium. The in vitro oocyte insemination was performed the day of ovulation induction of recipients. The blastocysts obtained were transferred 8 days after in vitro insemination, 15 llamas were selected as recipients, which were synchronized with CIDR for 8 days, 6 days after CIDR removal was induced ovulation in recipients with the application of 1 ml of GnRH previous ultrasound confirmation of the presence of a dominant follicle greater than 6 mm in diameter. 9 embryos alpaca and 6 embryos llama were transferred nonsurgically into the uterine horn ipsilateral to the corpus luteum. The pregnancy rate was assessed by ultrasound at 45 days after transfer. The results obtained were: for pregnancy rate, 33.3% (3/9) and 50% (3/6) for alpaca and llama embryos respectively; for birth rate 0.0% (0/9) and 16.7% (1/6) for alpaca and llama embryos respectively. An alpaca fetus and two fetus llama were aborted between 7 and 10 months of gestation, and only a llama gestation ends successfully, producing the first birth of the world of a offspring from in vitro embryos obtained from abattoir gametes in sudamerican camelids, demonstrating that it is possible to obtain viable offspring in these species using this biotechnology.

Keywords: In vitro fertilization, llamas, alpacas.

Financial support was provided by Proyecto FOCAM: Optimización de la fecundación in vitro para la conservación del material genético de las alpacas (*Vicugna pacos*) de la comunidad campesina de Carhuancho, Pilpichaca, Huaytará, Huancavelica.

{R–34} COMPARISON OF THE EFFECT OF TWO FREEZE-THAWING CURVES FOR PORCINE SEMEN. PRELIMINARY RESULTS

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ABSTRACT

Results obtained in fertility and litter size using frozen-thawed porcine semen are far from those obtained with natural service or artificial insemination of cooled semen. The objective of this study was to evaluate freeze-thawing of porcine semen comparing the traditional slow method to a rapid curve of temperature descent, using two cryoprotectants. Six males of proven fertility (n=6, r=2) were used. Semen was obtained using the gloved-hand technique and was transported to the laboratory at 17 ºC diluted in Androstar® plus. Samples were centrifuged and re-diluted in: a) 5% DMF, 11% lactose, 20% egg yolk, 0,5% Equex and b) 3% glycerol, 11% lactose, 20% egg yolk, 0,5% Equex. The semen was frozen in 0,5 ml straws up to a final concentration of 300x10 ^{6} sperm /ml using either: 1) a modified slow traditional Westendorff curve, or 2) a rapid curve according to Miragaya et al., (2001). Slow curve: semen was placed at 5 ºC for 2 hours followed by placing the straws horizontally 5 cm above the level of liquid nitrogen for 20 min and finally plunging the straws into liquid nitrogen. Rapid curve: straws were placed, submerged in a mixture of ethanol: 2-propanone, in a bronze canister with a graduated handle 6 cm over the liquid nitrogen vapors and temperature descent was carried out in two phases first a rate of 10–12 °C/min followed by a rate of 25–40 °C/min; and finally plunging the straws into the liquid nitrogen. In both cases thawing was carried out at 37 ºC during 1 minute. Sperm viability and acrosome status were evaluated using the FITC-PNA/PI stain. Cinetic motility parameters were evaluated using a CASA system (ISAS v1, Proiser, Spain). The results were analyzed using a factorial design (analysis of variance) with two factors (curve and crioprotectans), with two levels for each one and using the male as a blocking factor. Results: no interaction was observed between the two factors. No significant differences (p> 0,05) were observed between curves or between cryoprotectants for the percentage of live acrosome intact sperm (glycerol/rapid curve: 22 ±12; DMF/rapid curve: 22±9; glycerol/slow curve: 21±10; DMF/slow curve: 20±8). No significant differences (p> 0,05) were observed between curves or between cryoprotectants for total (TM) and progressive motility (PM) (glycerol/rapid curve: TM 22 ±13 and PM 12±7; DMF/rapid curve: TM 18±9 and PM 10±6; glycerol/slow curve: TM 19±7 and PM 10±6; DMF/slow curve: TM 27±14 and PM 10±6). Conclusion: taking into account that the results obtained did not differ, the rapid curve would be of choice as it is more practical, fast and manageable for fieldwork. In addition, the shorter exposure of sperm to the cryoprotectants could make it less toxic.

Key words: semen, porcine, cryopreservation.

{R-36} EFFECT OF CO-CULTURE WITH GRANULOSA CELLS ON THE DEVELOPMENT OF ALPACA (vicugna pacos) EMBRYOS PRODUCED BY IN VITRO FERTILIZATION

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ABSTRACT

The co-culture is a technique that could favor the development of the embryo as it provides nutrients and releases growth factors that could stimulate development in vitro to the blastocyst stage. The objective of this work was to evaluate the effect of co-culture with granulosa cells on rates of development of alpaca (Vicugna pacos) embryos produced by in vitro fertilization. Samples of ovaries and testes of alpacas were recovered in the slaughterhouse of Huancavelica and transferred in thermos to the Laboratory of Reproductive Biotechnologies of the National University of Huancavelica within two hours after the slaughter of animals. Cumulus oocyte complexes (COCs) were recovered by aspiration from ovarian follicles of 3-6 mm diameter. Were selected alpaca COCs of category I and II and were in vitro matured for 40 hours in an incubator with an atmosphere of 5% CO2 air, 99% relative humidity and a temperature of 38 °C. Then, COCs matured were transferred to in vitro fertilization FERT-TALP medium and inseminated with sperm recovered from the cauda epididymis (3 x 106 sperm/ml) which had between 70 - 80% motility. For the recovery spermatozoa SPERM-TALP medium was used and for the selection of motile spermatozoa swin up technique was used. Before in vitro insemination, spermatozoa were capacited for 30 minutes in FERT-TALP medium. Culturing of granulosa cells was obtained from the in vitro maturation plate after 40 hours, where those cells that had formed monolayer were trypsinized for a period of 2 minutes and recovered by centrifugation. For the co-culture of cells and embryos, Was used a cell suspension of 10 ul in medium SOF-IVC supplemented with 20% fetal bovine serum and glucose, after two hours of culture, assumptions embryos were transferred to the plate containing granulosa cells and maintained for 7 days in the cell culture chamber, where cleavage rates and blastocyst were evaluated at 2 and 7 days, respectively making changes of SOF – IVC médium each 24 hours. The treatments were, T1: with co-culture and T2: without co-culture. For statistical analysis Completely Random Design was used and the Tukey test was used to test the difference between averages. The results obtained were: for segmentation rate 31.6% and 33.2% for T1 and T2 respectively, for blastocyst rate 10.2% and 13.4% respectively. No statistical differences ($P > 0.05$) in both variables evaluated were found. In conclusion there is no effect of co-culture with granulosa cells on the development of in vitro embryos of alpaca.

Keywords: Granulosa cell, co-culture, alpaca, embryos.

Financial support was provided by Proyecto FOCAM: Optimización de la fecundación in vitro para la conservación del material genético de las alpacas (*Vicugna pacos*) de la comunidad campesina de Carhuancho, Pilpichaca, Huaytará, Huancavelica.

{37} HISTOLOGICAL AND HISTOCHEMICAL ANALYSIS OF THE OVIDUCTAL MUCOSA OF ALPACA (v*icugna pacos*). COMPARISON BETWEEN DEVELOPING AND ADULT FEMALES

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ABSTRACT

The oviduct plays a crucial role in several reproductive events, like the transport and maturation of gametes fertilization and early embryo development. However, the histophysiological features of alpaca oviduct still unknown. The aim of this work was deepen the study of the histological and histochemical features of the epithelium of the alpaca oviductal mucosa, in developing and adult females. We worked whit oviduct samples from developing (less than 2 years) (n=6) and adult (n=6) females alpaca (*Vicugna pacos),* wich were collected in slaughterhouse from Pilpichaca, Ayaviri and Nuñoa, Perú. Samples were fixed into buffered 10% formalin, were cut in the segments: ampulla, isthmus, utero-tubal junction (UUT), papilla, and then were processed to obtain paraffin slices. Hematoxilyn and Eosin staining was made for the descriptive epithelium histological study. For the histochemical study the stainings of PAS, to reveal neutral mucopolysaccharides, Alcian Blue (AA), to reveal acid mucopolysaccharides, and Oil Red, to reveal lipids, were made. The observation was carried out with a light microscope (Leica DM4000B), whit a digital camera (Leica DC380). We used the HSCORE method with 4 levels for the histocheimical analyses and Chi Square test for the statistical analysis, all results were considered to be statistically significant at $p \leq 0,1$. Oviductal epithelium ranging from the simple columnar to pseudostratified, ciliated. It presents ciliated cells and secretory type. The latter have vacuoles. Assessment epithelium by PAS and AA determined significant differences in the number of labeled cells among segments in both developing females ($p<0.1$) and adults ($p<0,1$). By analyzing the different segments, a greater amount of secretory vacuoles was observed, mainly in the ampoule, both adult alpacas ($p<0,1$) and developing ($p<0.1$). The other aspect analyzed was the presence of caveolar type glands in the epithelium, which were observed with lower levels of development and lower presence in females compared to adults. The caveolar vacuolated epithelium is primarily in adult isthmus and ampoule, while in alpacas variable degree of vacuolization was observed in regions and without vacuolization in the isthmus and ampoule. Vacuolated cells in caveolae, PAS and AA negative but positive Oil Red were observed. Negative cells are also observed at three techniques. Also were observed significant differences when comparing each technique on each oviductal segment between developing and adult alpacas ($p <0,1$) differences. Findings suggest variations in the epithelium of the oviductal mucosa among the oviductal segments from developing and adult alpacas, as well as variations in the production and secretion of acid and neutral glycoproteins, and glycolipids, which could be related to the organ physiology.

Keywords: oviduct, histology, histochemistry, Vicugna Pacos

{R-44} TRIS EXTENDER WITH QUAIL EGG YOLK ON CYTOPLASMIC MEMBRANE INTEGRITY OF EPIDIDYMAL SPERM REFRIGERATED CREOLES BULLS POST-MORTEM

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ABSTRACT

Conservation of genetic diversity, requires the use of several reproductive biotechnologies, such as conservation of gametes. Recovery and cryopreservation of epididymal sperm may be the last chance for the conservation of male sperm with high genetic value or germplasm. Sperm refrigeration allows a basic conservation and evaluate the properties of the quail egg yolk for its content of polyunsaturated fatty acids which prevents thermal shock and and greater stabilization of the sperm plasma membrane. The aim of the study was to evaluate the integrity of the cytoplasmic membrane of epididymal sperm of Creoles bulls postmortem, diluted with Tris more quail egg yolk (YHC). It is distributed in three treatments, epididymal sperm diluted with Tris more YHC 10, 15 and 20%, each group was evaluated at 0, 24 and 48 h of refrigeration maintained at 4 °C. It was used epididymis of slaughterhouse (n = 30), of Creoles bulls greater age 3 year, immediately post-mortem was used transported keeping temperature between 25 to 30 °C about 60 min. It was collected sperm from the cauda epididymis using the technique of retrograde flow with 3 mL of dilutor Tris more YHC according to the treatments, then around 15 min of collected, motility was evaluated, with individual motility to 70%, were subjected to test endosmosis (hypoosmotic - HOST), incubated for 30 min at 37 °C in sodium citrate plus fructose solution (100 mOsm / L). The test HOST was performed using 0.9 mL of hyposmotic solution and 0.1 mL of diluted sperm and then added 0.1 mL of 4% formaldehyde. They were counted 200 sperm cells by microscopy at 100 x. Positivity endosmosis test was considered when sperm cells showed edema, evidenced by winding tail. Analysis of variance was performed using the GLM procedure of SAS. For positivity to the test of epididymal sperm endosmosis, there was no interaction between factors refrigeration time and concentration of Tris dilutor more YHC (P = 0.6074). For the concentration factor, the means were similar (P = 0.9672); 72.7 ± 12.7, 73.3 ± 13.9 and 73.9 ± 14.7% for 10, 15 and 20% Tris more YHC dilutor. The refrigeration time factor affected (P = 0.0001) the integrity of the cytoplasmic membrane of epididymal sperm was found: 90.9 ± 4.0, 74.1 ± 9.2, 61.5 ± 8.1% positivity to test endosmosis for 0, 24 and 48 h of refrigeration. In conclusion. that the functional integrity of the cytoplasmic membrane of the epididymal sperm of Creoles bulls post-mortem, is affected by the refrigeration time, however, the addition of Tris extender with YHC does not affect it.

Keywords: hyposmotic test, sperm, refrigeration, bovine.

{45} EFFECT OF L-CARNITINE AND PYRUVATE ON EQUINE SPERM MAINTANIED AT 5 °C AND 15 °C DURING 24 H. PRELIMINARY RESULTS

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ABSTRACT

Spermatozoa from many stallions evidence a high susceptibility to temperature descent and the consequent peroxidation that is produced, factors which negatively affect their transport at 5 °C. For this reason the aim of this study was to evaluate if the addition of L-carnitine and pyruvate to two transport extenders is able to maintain sperm parameters for 24 h at 5 °C and 15 °C. Semen was obtained from 3 stallions (n=3; r=2) using an artificial vagina. After routine evaluations, the samples were subdivided into the following extenders: 1) Kenney extender (K); 2) K extender with the addition of 6 mM L-carnitine and 6 mM pyruvate (K+); 3) modified Kenney extender (KMT); 4) KMT with the addition of 6 mM L-carnitine and 6 mM pyruvate (KMT+). After dilution, samples were maintained in either an Equitainer or a Botubox, where a controlled temperature descent was carried out (to 5 °C and 15 °C respectively). At time 0 and after 24 h of cooling, the following sperm parameters were evaluated: total and progressive motility, both subjectively between slide and coverslip on a warm stage (37 °C) using phase contrast microscopy and objectively, using Computer Assisted Semen Analysis (CASA). Viability and acrosome status (FITC -PNA-PI), membrane function (HOS test), and DNA with Toluidine blue stain (TB) and Sperm Chromatin Dispersion assay (SCD) were also evaluated. Each temperature was individually analyzed using a factorial design with a 5% significance level. No interactions were observed. At 24 h, a significant effect both of time and treatment was observed, with the K+ extender preserving progressive motility best at both temperatures ($p=0,0094$ and $p=0,0270$). In addition, both temperatures showed a significant ($p<0,05$) decrease of viable acrosome intact sperm, with the K+ extender the only one to be not significantly different from time 0 ($p= 0,6980$ and $p=0,7435$ respectively). Membrane function decreased at 24 h, with no significant differences being observed between the extenders assayed ($p>0,05$). With regard to the DNA, no significant decrease of chromatin condensation was observed at either temperature or for any extender ($p>0,05$). Nevertheless, at 24 h, an increase in DNA fragmentation was observed, with the K extender maintaining DNA integrity better at 5 °C and both extenders with L-carnitine and pyruvate (K+ and KMT+) maintaining DNA integrity better at 15 °C ($p<0,05$). To conclude, for the moment, the best results for maintaining the majority of the sperm parameters for 24 h at either 5 °C or 15 °C were obtained using Kenney extender with the addition of L-carnitine and pyruvate. Current studies are under way to evaluate 22 °C as an alternative temperature for maintaining equine semen for transport.

Keywpords. Equine, sperm, refrigeration, L-Carnitine, pyruvate

{46} COMPARISON ULTRASONOGRAPHIC OF DYNAMICS FOLLICULAR AND CORPUS LUTEUM IN COWS AND HEIFERS SUBJECTED TO A ESTRUS SYNCHRONIZATION PROTOCOL

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ABSTRACT

The aim of this study was to compare ultrasound characteristics of follicular dynamics and corpus luteum (CL) in cows and heifers under a protocol of estrus synchronization and timed artificial insemination (TAI). A variety of factors may influence the likelihood of pregnancy at the time of artificial insemination (AI) including control of the dynamics of the follicular wave and time of estrus and ovulation, steroidogenic capacity, capacity of the ovulatory follicle, oocyte competence, ability the uterus for an embryo and function of CL after ovulation. the characteristics of follicular dynamics and CL of 36 animals between cows and heifers of the Brown Swiss breed subjected to estrus synchronization protocol with fixed-time AI with intravaginal device (Pro-ciclar® contains 750 mg of Progesterone) was determined. Sonographic evaluation was conducted with a team CHISSON D600VET with a frequency of 7.0 MHz in 4B mode, follicular activity was evaluated (insert the device and application of 2 mg of estradiol benzoate) on the first day (Day 0) or synchronization start were evaluated ovarian status then the Day 8 were evaluated by ultrasonography and 10 (post synchronization start) to determine the rate of follicular growth (Day 8 the device was removed, he applied eCG and Prostaglandin F2a) Observed a rate follicular growth of 2,19 ± 1,13 mm in cows and 1,93 ± 1,13 mm in heifers ($p < 0,05$) and a maximum diameter of follicle in cows and heifers of 15,91 ± 2,61 and 12,77 ± 1,33 mm ($p < 0,05$) respectively which was assessed on Day 10 (when the IA and the application of GnRH) the ovulation rate was 66.7% in both groups, performing ultrasounds every 12 hours from Day 10 to 12 (the start of estrus synchronization); regarding the characteristics of the CL evaluated on Day 15 for cows were 17,92 ± 2,85 mm and 11,32 ± 1,96 mm to heifers ($p < 0,05$) observed an alternation in ovarian function. The results reported similar behavior on follicular dynamics in the group of cows and heifers but not compared to the same altitude studies also reported in Brown Swiss cows. However pregnancy rates was 44,4% and 66,7% in cows and heifers respectively ($p < 0,05$). The sonographic features of follicular dynamics and CL shows differences in the two groups except in the ovulation rate, noting that the group of heifers reported higher pregnancy rate compared to the cows.

Keywords: Ovary, follicular dynamic, ultrasonography, corpus luteum.

{R-48} ANNUAL VARIATION OF ESTROUS MANIFESTATION AND OVULATION RATE IN RIPOLLESA EWES

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ABSTRACT

Studies on reproductive seasonality in sheep are based on the characterization of the sexual activity of a breed, for a geographical area and a specific production system. This knowledge allows to implement management practices and to introduce reproductive techniques in order to improve production efficiency. Because of scarce information on this subject, the aim of this study was to determine the annual variation of estrus manifestation and ovulation rate in Ripollesa breed ewes. A total of 16 adult and multiparous ewes recently lambed (1-3 months) and 2 vasectomized rams were maintained during the whole year under semi-intensive and natural photoperiod conditions. The experiment was conducted under semi-stabling conditions with natural grazing. The methodology proposed by Chemineau and Thimonier (1986) was utilized by carrying out the following activities: Monthly record of liveweight and corporal condition. Daily detection of estrus by recording the ewes marked by vasectomized males. Exploratory laparoscopy at day 7 after estrus presentation in order to determine the number of corpora lutea per ewe. order to detect the presence of silent ovulations (no symptoms of estrus), blood venipuncture every 7 days was done to determine plasma progesterone concentration by radioimmunoassay (RIA). Percentages of ewes in estrus during summer (July-September), autumn (October-December), winter (January-March) and spring (April-June) were 94, 75, 31 and 44%, with a mean ovulation rate of 1.6, 1.5, 1.4 and 1.1 corpora lutea/ewe, respectively. Cyclic estrous activity began in 43.8% of the ewes in autumn, 43.8% in winter and 6.3% in spring. A 6.3% of ewes displayed permanent cyclic estrous activity throughout the year. Annual values of sexual inactivity (anestrus) observed according to their duration (60 days, between 61-120 days and 121-180 days) were 18.8, 43.8 and 37.5%, respectively. Progesterone analysis evidenced that 18.8% of ewes presented silent ovulations in spring, 4.4% in winter and only 2.1% in autumn, with no silent ovulation presentations in summer. It is concluded that Ripollesa ewes have a high cyclic estrous activity in the summer-autumn period that reduces considerably in the winter-spring period, resembling data from other sheep breeds of the peninsula Ibérica. It is established that this breed presents an important monthly variation in the ovulation rate throughout the year and that, by carrying out matings during the period of greatest estrus manifestation (summer and autumn), it would present a greater probability of obtaining twin births, highlighting its reproductive potential. Future studies should assess the possibility of increasing the number of ewes with cyclic estrous activity throughout the year in order to design new strategies to increase lamb production.

Keywords. Reproductive seasonality, ovine, ovulation rate, Ripollesa