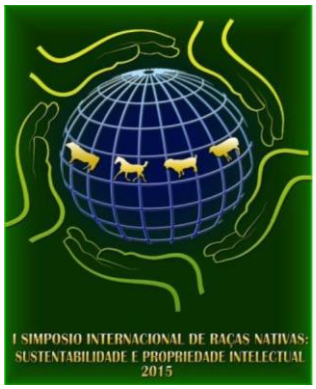

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Preliminary results in the application of advanced embryo transfer technologies for the genetics improvement of Morada Nova sheep

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Abstract: Multiple ovulation and embryo transfer (MOET) and Laparoscopic Ovum Pick-Up (LOPU) followed by In Vitro Embryo Production (IVEP) were tested in the Morada Nova breed to explore its potential for genetic improvement of established flocks as well as for exporting the breed to other regions of and/or other tropical countries where it is believed that the breed can make a significant contribution to sheep production. Our results show that an average of ~5 transferable embryos/MOET donor and ~3 transferable embryos/LOPU donor can be produced in Morada Nova sheep using standard protocols established in other breeds, although this numbers can probably be improved by working in the fall. In addition, MOET embryos subjected to freezing and thawing prior to transfer into recipients resulted in a ~50% pregnancy rate also suggesting that the technology would be very useful for exporting Morada Nova genetics.

Keywords: in vitro, freezing, flushing, laparoscopic ovum pick-up

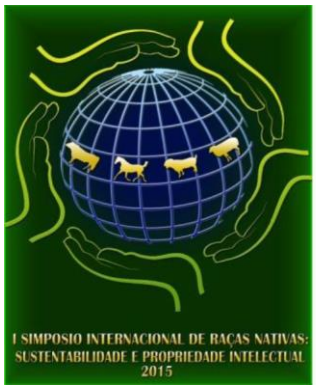
Resultados preliminares da aplicação de tecnologias avançadas de transferência de embriões para o melhoramento genético de ovelhas da raça Morada Nova

Resumo: A ovulação múltipla e transferência de embriões (MOET), e a aspiração de ovócitos por laparoscopia (LOPU) seguida pela Produção In Vitro de Embriões (IVEP) foram testados na raça Morada Nova, a fim de explorar seu potencial para o melhoramento genético dos rebanhos estabelecidos, bem como a exportação de material genético desta raça para outras regiões e/ou países tropicais, onde o Morada Nova poderia contribuir significativamente para a ovinocultura local. Os nossos resultados mostram que uma média de ~ 5 embriões viáveis / doadora MOET e ~ 3 embriões viáveis / doadora LOPU podem ser produzidos em ovelhas Morada Nova utilizando protocolos padrões estabelecidos para outras raças, embora esperamos que resultados melhores poderão ser obtidos, se o trabalho for realizado durante o outono. Além disso, os embriões MOET submetidos ao congelamento e descongelamento antes da transferência para receptoras resultou em uma taxa de gestação em torno de 50%, o que sugere que a tecnologia pode ser muito útil para a exportação de material genético da raça Morada Nova.

Palavras-chave: in vitro, congelamento, coleta ovócitos, laparoscopia

Introduction

Morada Nova sheep is a breed developed in northeastern Brazil in the state of Ceará, with the characteristics of having no wool and having good quality leather. It´s a very prolific sheep, very rustic to suit most arid regions, and it plays an important social role by providing protein foods to rural populations of these regions. Embryo transfer technologies are essential tools for genetic improvement as well as for the purpose of exporting breeds and lineages to regions where they are exotic but could adapt easily and be productive. Frozen embryos from adult Morada Nova ewes could be moved from one region to another, implanted in local recipients to ensure adaptation and disease resistance of the newborns, and the progeny could be subjected to LOPU at Prepubertal ages and shorten the generation interval and move faster towards the establishment of full size herd. In order to validate the concept, we conducted some preliminary in vivo and in vitro embryo production as well as some embryo freezing efforts during the months of July/August in Itapira, SP (winter).



Materials and Methods

In Experiment 1 we explored the use of superovulation and embryo freezing in 8 adult Morada Nova ewes of 1.5 to 5 years of age. The embryo donors were estrus synchronized by means of CIDR devices inserted for 14 days, with the original CIDR been exchanged for a new CIDR after 7 days at which point also a luteolytic dose of 250 µg cloprostenol (Ciosin, MSD Lab, Brazil) was injected. Superovulation was stimulated by means of 8 decreasing dose injections of FSH (Folltropin, Bioniche Labs, Canada) administered at 12h intervals starting 48h prior to CIDR removal and totalling 256 mg FSH per ewe. In order to promote and synchronize multiple ovulations, a dose of 50 µg GnRH (Fertagyl, Intervet Lab, Netherlands) was administered to donors 36h after CIDR removal. Fixed-time laparoscopic insemination was used for breeding of donor ewes. Some received a single insemination with fresh semen about 40h after CIDR removal, while donors bred with frozen semen were inseminated twice, at 40 and 46h post-CIDR withdrawal. Prior to surgery the animals were deprived of food for 24h and water for 12h. An exploratory laparoscopy was performed in order to assess the response of each donor and only donors with at least 3 morphologically sound corpora lutea were selected for embryo recovery. Embryo collection was performed through mid-ventral laparotomy under general anesthesia. The uterine horns were exteriorize through an incision of approximately 5 cm, immediately cranial of the mammary gland. A 20G intravenous catheter was inserted in the tubal third of the uterus and a 10Fr. silicon Foley catheter was inserted at the base of the uterine horn. Both horns were flushed by injecting 40 mL of commercial flushing medium through the IV catheter and recovering it through the Foley into a 50 mL Falcon tube. The contents of the collection tube were then observed under a stereomicroscope in order to find and grade the recovered embryos. Only grade 1-2 embryos in the compact morula/blastocyst stage were selected for freezing. Commercial 1.5M ethylene glycol freezing medium was used (Vigro Freeze Plus, Bioniche Lab, Canada). Briefly, the embryos were washed in holding medium and then equilibrated in freezing medium for up to 10 min (not less than 5, not more than 10), loaded in 0.25 cc sterile straws previously identified with donor and male information and transferred into the freezing machine previously equilibrated at -6°C. After 1 minute the straws were seeded by touching with a forceps previously immersed in liquid nitrogen (LN2). Finally the machine program brought the temperature down to -35°C at a rate of 0.5°C/min and the straws were finally immersed in LN2 and stored in properly identified goblets inside a LN2 tank. Frozen-thawed embryos were transferred into recipients of Morada Nova breed that were synchronized to be on day 7 of their cycle using intravaginal sponges containing 60 mg medroxyprogesterone acetate for 2 weeks and 500 IU PMSG at sponge removal. For embryo transfer the tip of the horn ipsilateral to the CL was exteriorized through a small mid-ventral laparotomy (2 cm), under general anesthesia and laparoscopic visualization, followed by implantation into the uterine horn using a Tomcath® catheter loaded with the embryo. In Experiment 2 we explored the use of Laparoscopic Ovum Pick-Up (LOPU) and In Vitro Embryo Production (IVEP) in 12 Morada Nova ewes of various ages ranging from 8 months to 5 years. Donors were estrus synchronized by means of intravaginal sponges for 2 weeks, and received a total of 100 mg FSH in three decreasing dosage injections at 12h intervals starting 36h prior to LOPU. Prior to surgery the animals were deprived of food for 24h and water for 12h. LOPU was conducted under general anesthesia with the animal lying on a cradle with a 45 angle to facilitate the visualization of reproductive organs. The procedure utilized has been described in full elsewhere (1-2). Briefly, looking through the laparoscope, the ovarian surface was exposed by pulling from the fimbria with an atraumatic grasping forceps, and the follicle contents were aspirated with a pipette that has a 20G needle glued to the tip at one end and is connected to a collection tube and a vacuum pump on the other end. Media and procedures for IVM, IVF and IVC are described elsewhere (1-2). Briefly, at the end of LOPU the contents of the collection tube were observed under the stereomicroscope, and the cumulus-oocyte complexes (COC's) that were recovered were washed in medium prior to transfer into 50 µL drops of IVM medium under mineral oil and placed in the incubator at 38.5°C and 5% CO2 for 22-24h to achieve maturation. Subsequent to that the oocytes were inseminated with semen that was previously capacitated by refrigeration in partially skimmed milk overnight and washed twice by centrifugation in IVF medium. The insemination dose was 100,000 sperm per IVF drop. Approximately 15-20h after IVF the presumptive zygotes were transferred to IVC plates composed of 40 µL drops of IVC medium under mineral oil and incubated at 38.5°C in an atmosphere of 5% O2, 5% CO2 and 90% N2. Development was evaluated after

culture for 5-7 day. During this period embryos were evaluated and moved to new drops of IVC medium every 48h.

Results and Discussion

In experiment 1, only 5/8 ewes responded properly to superovulation and were flushed. A total of 35 ova were recovered from these 5 donors, of which 26 were of grade 1-2 and eligible for freezing, resulting in an average of 3.2 good quality embryos per donor superovulated and 5.2 good quality embryos per donor flushed. There was a trend for a higher percentage of non-responders in the younger donors of <18 months of age (2/3, 67%) than the older donor (1/5, 20%). To this date, 18 of the 26 embryos frozen have been transferred into 11 recipients in sets of 1-2 embryos/recipient and this has resulted in 6 recipients pregnant (54.5%). In experiment 2, a total of 81 oocytes were recovered from 12 donors (average 6.7 oocytes/donor) of which 57 cleaved after IVF (70%) and 37 developed to the compact morula/blastocyst stage (46%) after 5-7 days in culture. As shown in Table 1 there was a trend for oocytes of adult animals (>18 months of age) having similar cleavage rate but higher developmental competence compared to oocytes from younger donors.

Table 1: Effect of age on oocyte recovery, cleavage and embryo development

Age of donors	n	Oocytes recovered	Cleaved (%)	Morula/Blastocyst (%)
>18 months	7	44	33 (75%)	25 (57%)
<18 months	5	37	24 (67%)	12 (32%)

Conclusions

Our preliminary work shows good potential for the utilization of embryo transfer technologies in the applications suggested in the introduction. Multiple ovulation and embryo transfer resulted in an average of >5 transferable/freezable embryos per donor flushed, an average that is considered to be commercially acceptable, comparable to results reported by others (LOPES JE et al., 2006) and probably easily improvable considering that our work was conducted in the winter when conditions were not at best. The fact that we were able to achieve a >50% pregnancy rate in recipients transferred with frozen/thawed embryos is also a good indication of the role of embryo transfer technologies for moving Morada Nova genetics to regions where the breed can be used to substantially improve productivity of local sheep business, including other tropical countries. In vitro embryo production has also shown good preliminary data although some improvements at the level of average number of oocytes/donor would be desirable (publications show average of ~10 oocytes/donor in European breeds). Potentially this is also partly attributable to conducting the experiment during the winter, but further research should be pointed towards the optimization of hormonal treatment of donors to maximize oocyte yields, as well as towards the production of embryo transfer data to assess the potential of LOPU-IVEP in the Morada Nova breed. It is noteworthy that as analyzed in detailed in prior publications (BALDASSARE et al., 1996; BALDASSARE H., 2012), LOPU-IVEP offers the advantage of allowing to practice the procedure in the same animal more times and more often than standard MOET, thereby having the potential of yielding more progeny in the reproductive life of genetically superior animals. To the best of our knowledge, this is the first report of LOPU-IVEP in the Morada Nova breed.

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