Trop Anim Health Prod (2017) 49:1511–1516
DOI 10.1007/s11250-017-1356-y

REGULAR ARTICLES

Reproductive outcomes of anestrous goats supplemented with spineless *Opuntia megacantha Salm-Dyck* protein-enriched cladodes and exposed to the male effect

Cesar A. Meza-Herrera[1,2] · **Omag Cano-Villegas**[1,3] · **Arnoldo Flores-Hernandez**[1] · **Francisco G. Veliz-Deras**[4] · **Guadalupe Calderon-Leyva**[4] · **Juan M. Guillen-Muñoz**[4] · **Cristina García de la Peña**[3] · **Cesar A. Rosales-Nieto**[5] · **Ulises Macias-Cruz**[6] · **Leonel Avendaño-Reyes**[6]

Received: 17 January 2017 / Accepted: 5 July 2017 / Published online: 13 July 2017

Abstract The possible influence of the "male effect" upon reproductive outcomes of adult anestrous goats under marginal rangeland conditions and supplemented with protein-enriched *Opuntia megacantha Salm-Dyck* was evaluated. Reproductive variables included: estrus percentage (EST, %), estrus latency (ESL, hours), ovulation percentage (OP, %), ovulation rate (OR, units), average largest follicle at ovulation (LFO, mm), largest corpus luteum (LCL, mm), embryo number (EBN, units), and embryo implantation percentage (EIP, %). During early May, anestrous mix-breed adult goats (Criollo x Alpine-Saanen-Nubian; n = 38, 26° N) were randomly distributed to (1) *Control* (CC; n = 12), (2), *Non-enriched Opuntia* (NEO; n = 14), and (3) *Protein-enriched Opuntia* (PEO; n = 12). Neither LW ($P > 0.05$) nor BCS ($P > 0.05$) or any of the evaluated ovarian variables differed ($P > 0.05$) among treatments; EST = 89.66%, ESL = 53.66 h, OP = 70.33%, OR = 1.07 units, LFO = 4.5 mm, LCL = 9.6 mm, EBN = 0.94 embryos, and EIP = 48.66%. Irrespective of nutritional supplementation regime, all goats denoted an increased response to the male effect just in the middle of the anestrous season and managed under marginal grazing conditions during the dry season (May to June; 26° N). The use of the male effect successfully invoked neurophysiological pathways to re-activate ovarian follicular and luteal pathways during the natural anestrous season in the female goat. Yet, such successful physiological scenario was not equally exerted to promote an increased embryo implantation rate; this issue claims further consideration. Therefore, it is essential to align not only the peri-conceptional but also the peri-implantation stages to the best suited environmental conditions in the rangeland, in order to increase both reproductive and economic efficiency while promoting sustainability in those rangeland-based marginal goat production systems.

Keywords Goats · Male effect · *Opuntia* · Reproductive efficiency · Targeted supplementation

✉ Cesar A. Meza-Herrera
cmeza2020@hotmail.com; cmeza2000@gmail.com; http://www.researchgate.net/meza-herrera

[1] Regional Universitary Unit on Arid Lands, Chapingo Autonomous University, Bermejillo, 35230 Durango, Mexico

[2] Unidad Regional Universitaria de Zonas Áridas, Universidad Autónoma Chapingo, Bermejillo, 35239 Durango, Mexico

[3] Faculty of Biological Sciences, Juarez University of the State of Durango, Gomez Palacio, 35010 Durango, Mexico

[4] Department of Veterinary Sciences, Antonio Narro Agricultural Autonomous University, 27054 Torreón, Coahuila, Mexico

[5] National Institute for Forestry, Agriculture and Livestock Research, 78431 San Luis Potosí, SLP, Mexico

[6] Institute of Agricultural Sciences, Baja California Autonomous University, 21705 Ensenada, Baja California, Mexico

Introduction

A recurrent scenario observed in animal production systems under rangeland conditions is the absence of a continuous food supply across the year, limiting their productive efficiency (Gonzalez-Bulnes et al. 2011; Meza-Herrera and Tena-Sempere 2012). In different production systems under arid and semiarid conditions around the world, there is a notable abundance of native cacti (*Opuntia* spp) which has a rich composition in polyphenols, vitamins, polyunsaturated fatty acids, and amino acids (El-Mostafa et al. 2014). Nonetheless, the cladodes of *Opuntia* (prickly pear cactus) have been generally characterized because of its quite reduced protein content (Akanni et al. 2015). Interestingly, the protein-enrichment of *Opuntia* cladodes has shown to increase the crude protein content from 4 up to 30%, throughout a semisolid fermentation bioprocess (Díaz-Plascencia et al. 2012). Because of the continuous increase in the price of concentrates along with an augmented use not only of cereals but also other cellulosic products by the bio-fuel industry, a quite complicated perspective emerges, a situation that conspires against the sustainability of the most vulnerable segment of the livestock sector, both from a biologic and economic stand point (Mullins et al. 2014; Ren et al. 2014).

Besides, the use of socio-sexual cues such as the male effect is an interesting strategy to improve reproductive outcomes since it represents an important approach to induce ovarian cyclicity (Martin et al. 1986; Flores-Najera et al. 2010; Luna-Orozco et al. 2012). Yet, the female response to the male effect is modulated and shaped by several environmental factors such as the nutritional status (Gelez and Fabre-Nys 2004). Certainly, a low nutrition level reflected by a decreased body condition score of either males or females affects in a paramount fashion reproductive function (Urrutia-Morales et al. 2009; Flores-Najera et al. 2010). Based on such rationale, our working hypothesis considered that targeted supplementation of protein-enriched *O. megacantha Salm-Dyck* cladodes would speed up ovarian function and reproductive outcomes in previously anestrous adult goats managed under marginal rangeland-grazing conditions and exposed to the male effect; therefore, this study was designed to test such hypothesis.

Material and methods

General

All procedures and methods used in this study regarding the use and care of animals were carried out in accordance with accepted international (FASS 2010), national (NAM 2002) animal use and care guidelines, with institutional approval 14-510-4002.

Location, environmental and rangeland conditions, animal management

The study was carried out in a commercial farm under extensive conditions in northern Mexico (26° 23′ N, 103° 47′ W, 1117 m elevation). The rainy season extends from June to October, and mean annual rainfall and temperature are 225 mm and 24 °C. While relative humidity varies from 26.14 to 60.69%, the photoperiod varies from 13 h and 41 min (summer solstice, June) to 10 h and 19 min (winter solstice, December). The vegetation type is a highly degraded desert scrub and is characterized as Chihuahuan desert rangeland. While creosotebush (*Larrea tridentata* (*DC. Cov*)) dominates the grazing area, other important species include lechuguilla (*Agave lechuguilla Torr*), mesquite (*Prosopis glandulosa v. glandulosa*), and blue grama (*Bouteloua gracilis* (*Wild. ex Kunth*) (*Lag. ex Griffiths*). Stocking rate is approximately 1.5 ha per goat, above the rangeland carrying capacity. Total standing yield in this environment is around 2000 kg dry matter per hectare, with browse providing the bulk of available forage; goats graze on a deteriorated rangeland with a low forage production potential (Mellado et al. 2012). Goats graze mostly on rangelands although occasionally on crop residues such as corn, sorghum, and cotton, because of the irrigation district located in the area. Since all goats were taken to different grazing sites every day, walking approximately 5 km daily from the pen to the rangeland, grazing constrains can be considered negligible in goats that are taken daily to different sites (Mellado et al. 2012). During the spring-summer seasons, goats grazed the rangeland driven by a herdsman 9 h daily (10:00 to 19:00 h) and penned from 19:00 to 10:00 h. Goats spent the night in unroofed corral where they had free access to water and a commercial mineral-mix.

Animals and experimental treatments

Mix-breed adult non-pregnant, non-lactating, anestrous goats (Criollo x Alpine-Saanen-Nubian; n = 38) of known fertility were kept isolated from sight, sound, and smell of bucks at least 3 months before the onset of the trial. Thereafter, by middle May during the mid-anestrous season, goats were randomly distributed into three experimental groups: (1) Control (CC; n = 12; 41.3 ± 1.8 kg LW, 1.65 ± 0.05 units BCS, without feed supplementation), (2) Normal Opuntia (NEO; n = 14; 41.1 ± 1.75 kg LW, 1.57 ± 0.05 units BCS), and (3) Protein-enriched Opuntia (PEO; n = 12; 39.9 ± 1.7 kg LW, 1.60 ± 0.05 units BCS). Both the NEO and PEO goats were individually supplemented with 160 g day^{-1} from 0900 to 1000 h during a 10-day adaptation period. Such supplementation schedule was based on previous field observations where goats consume all the supplement if offered prior to grazing. Goats had free access to water and a commercial mineral-mix at the pen, during the evening-night hours and were not treated against internal parasites, since this is not a common health problem under this dry environment.

Experimental supplements and supplementation schedule

The experimental group PEO considered the protein-enrichment of cladodes throughout a semisolid fermentative process by mixing small slices of *Opuntia* cladodes inoculated with *Scharomyces cereveciae* (1%), urea (1%), and ammonium sulfate (0.1%) in a bioreactor (Nopafer-R, Lerdo Durango, Mexico) during a period of 20 h. Thereafter, the enriched cladodes were semi-dried at ambient temperature during 24 h. The chemical composition of both *Opuntia* treatments (NEO and PEO) is presented in Table 1. The three experimental groups were kept together during the day in the rangeland, while separated accordingly at the evening. Ten and 5 days prior exposure to the males, all goats were subject to an ultrasonographic scanning (USS) to confirm the anestrus status. In addition, 2 days after the USS, all goats received a single intramuscular injection of progesterone (20 mg; Fort Dodge, DF, Mexico) in order to reduce the occurrence of short luteal cycles as suggested by Chemineau et al. (2006). Thereafter, goats of the NEO and PEO groups received the same supplementation schedule during a 30-day post-adaptation period.

Table 1 Mean chemical composition (SD), dry basis, of *Opuntia megacantha Salm-Dyck* cladodes either protein-enriched (PEO) or non-protein enriched (NO) offered as supplement to adult mix-breed (Alpine-Saanen-Nubian x Criollo) female goats exposed to testosterone treated bucks and to an increased natural photoperiod (May–June; anestrous season) under semiarid-subtropical rangeland conditions in Northern Mexico (26° NL)

	NEO, fresh	NEO, dry	PEO, fresh	PEO, dry
DM, %	12.9	92.1	12.5	92.0
CP, %	6.4	4.9	29.8	20.5
NDF, %	21.3	14.7	18.3	17.5
ADF, %	19.7	11.9	16.6	17.9
NFC, %	43.8	53.3	24.4	33.9
TND, %	53.1	61.0	57.2	56.4
NEm, Mcal/kg DM	1.8	2.3	2.2	2.2
Ash, %	27.9	24.7	25.5	26.7

NEm was calculated using equations proposed by the NRC (2007)

Buck management: in search of the male effect

Once completed the adaptation period, on day 11, goats from the three treatments were exposed to six sexually experienced mix-breed dairy adult bucks (Alpine-Saanen, two per treatment, 3 to 4 years old) of proven fertility and libido. Males were kept in a ruffed cement floor pen (6 × 6 m) before breeding, with free access to alfalfa hay, water, and a mineral-mix. Previous to the contact with females, all bucks received an intramuscular injection of testosterone (50 mg, Testosterone, Lab Brovel, DF, Mexico) every 3 days × 3 weeks before the experimental breeding (Luna-Orozco et al. 2012). Thereafter, the bucks were kept in contact with the experimental females groups from 1900 to 0800 h daily.

Ultrasonographic evaluation of the ovary function and structures

The experimental breeding period started in early June and lasted 10 days. Daily occurrence of goats showing either estrus signs or copulation was recorded being defined such behaviors as occurrence of ovulation as a result of the male effect. Estrus was observed 1 hour twice per day (0800 and 1900 h) during the 10-day breeding period. The interval between the onset of joining and occurrence of estrus was also recorded. A transrectal real-time B mode USS (Aloka SSD 500 Echo Camera, Overseas Monitor Corp. Ltd., Japan) was performed during the first 6 days after male introduction to evaluate the dynamic of follicular growth and the time of ovulation. Males were removed from the experimental breeding 10 days after the onset of joining.

Thereafter, a third USS was performed to quantify the ovulation rate, measured as the number of corpora lutea present in each ovary on day 20 and confirmed on day 30, after male introduction. At this time, the *Opuntia* supplementation was concluded. A final USS was performed on day 45 to verify the implantation of embryos in endometrial tissue. All the four USS were performed by the same skilled operator. Ovaries were visualized at an image magnification of ×1.5, and the number and diameters of both follicles and corpus luteum observed in each structure were recorded and measured according to the procedures outlined by Dickie et al. (1999). The corpus luteum was identified on gray scale as hypoechoic area within each ovary; the size was calculated as the average of transverse, anteroposterior, and sagittal diameters. Ultrasonographic images were also recorded for retrospective analyses. Thereafter, embryo implantation was diagnosed 45 days post-male introduction. Ovarian function considered the response variables: estrus percentage (EST, %), estrus latency (ESL, hours), ovulation percentage (OP, %), ovulation rate (OR, units), average follicular (LFO, mm), and corpus luteum (LCL, mm) size. In addition, embryo number (EBN) and embryo implantation percentage (EIP) were also quantified. A schematic representation with the main activities performed during the experimental protocol is shown in Fig. 1.

Statistical analyses

Data on goats in estrus, ovulating, pregnant, abortions, and kidding were analyzed by categorical procedures using the GENMOD procedure of SAS with the LOGIT function. The only effect included in the model was the supplementation treatment, with each animal as a single experimental unit. When significant differences were found among treatments, the LSMEAN/DIFF procedure of SAS was used to compare the mean values. Analysis of variance (PROC GLM; SAS) was used for the interval between the onset of exposure, the occurrence of estrus, the length of estrus as well as for follicular, and corpus luteum size. The protected LSD procedure was used to compare means. Body condition score among

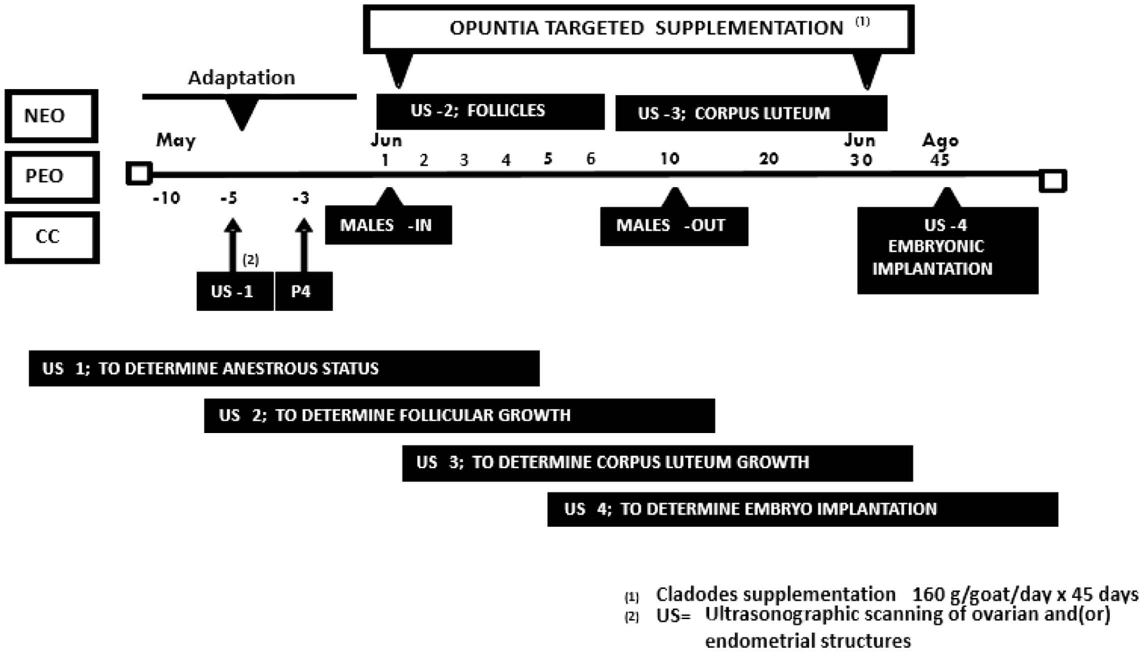

Fig. 1 A schematic representation of the experimental protocol of targeted supplementation with *Opuntia megacantha Salm-Dyck* cladodes either protein-enriched (PEO) or non-protein enriched (NEO) as well as not supplemented control (CC) to adult mix-breed (Alpine-Saanen-Nubian x Criollo) female goats exposed to testosterone treated bucks and to an increased natural photoperiod (May–June; anestrous season) under semiarid-subtropical rangeland conditions in Northern Mexico (26° NL). More details were previously described in the main body of the text

treatments was analyzed throughout Kruskal-Wallis test. All the analyses were computed through the procedures of SAS (SAS Inst. Inc. Version 9.1, 2004, Cary, NC, USA); the significance level was set at $P < 0.05$.

Results

At the beginning of the experimental breeding, no differences among treatments were observed neither for LW ($P > 0.05$) nor for BCS ($P > 0.05$). In addition, none of the evaluated ovarian variables differed ($P > 0.05$) among treatments: EST = 89.66%, ESL = 53.6 h, OP = 70.3%, OR = 1.07 units, LFO = 4.5 mm, LCL = 9.6 mm, EBN = 0.94 embryos, and EIP = 48.6%. Similarly, neither LW (41.54 ± 1.75 kg) nor BCS (1.46 ± 0.10 units) differed among treatments at the end of the experimental period. Besides, the presence of short estrus cycles was not observed among treatments ($P > 0.05$). A summary of data regarding the response variables according to the experimental treatments is presented in Table 2.

Discussion

The obtained results from our study give no evidence to support our working hypothesis. Certainly, neither LW nor BCS or any of the evaluated ovarian variables differed among treatments. Nonetheless, since previous to both *Opuntia* supplementation and male exposure, all goats depicted a definitive anestrous status, results of the study claim in particular importance that all goats denoted an increased response to the male effect just in the middle of the anestrous season. The last

Table 2 Least square means regarding live weight (kg), body condition score (units), reproductive, and ovarian parameters from adult mix-breed (Alpine-Saanen-Nubian x Criollo) female goats supplemented with *Opuntia megacantha Salm-Dyck* cladodes either natural (NEO) or protein-enriched (PEO) or non-supplemented control (CC). Adult goats were exposed to testosterone treated bucks and to an increased natural photoperiod (May–June; anestrous season) under semiarid-subtropical rangeland conditions in Northern Mexico (26° NL)[a]

	NEO	PEO	CC	S.E.[b]
LW-initial, kg	41.1[a]	39.9[a]	41.3[a]	1.8
BCS-initial, units	1.5[a]	1.6[a]	1.6[a]	0.05
LC-final, kg	41.0[a]	42.8[a]	40.7[a]	1.7
BCS-final, units	1.4[a]	1.4[a]	1.5[a]	0.10
Estrus, %	92.8[a]	85.7[a]	92.3	8.4
Latency, h	47.1[a]	48.0	66.4[a]	10.0
Ovulation, %	64.2[a]	78.5[a]	69.2[a]	12.9
Ovulation rate, units	1.14[a]	0.85[a]	1.23[a]	0.24
Ovulatory follicle, mm	3.5[a]	5.5[a]	4.5[a]	0.9
Corpus luteum (largest), mm	8.8[a]	9.9[a]	9.0[a]	1.8

[a] No differences among treatments occurred regarding any of the response variables evaluated ($P > 0.05$)

[b] Most conservative standard error is presented

was observed irrespective of the nutritional supplementation regime, and in goats managed under grazing conditions during the dry season while facing an increased photoperiod (May to June; 26° N). Worth mentioning, the reduced proportion of implanted embryos unequivocally vanished any potential benefit obtained by the upshot of the male effect, and in goats just in the middle of the natural anestrous season exposed to quite high environmental temperatures (>43 °C).

Our results do not support previous findings in which *Opuntia* supplementation, the so-called cactus effect, demonstrated a positive effect upon reproductive outcomes in sheep (Rekik et al. 2010; Sakly et al. 2012). More intriguing yet, our results did not agree with previous reports denoting a positive result exerted by *Opuntia* supplementation upon ovarian function, more precisely upon growth and developmental competence of preovulatory follicles (Rekik et al. 2012; Sakly et al. 2014) and ovulation rate (Rekik et al. 2012). Interestingly, and contrary to these results, administration of *Opuntia dilleni phylloclade* extracts to male rats reduced in a significant fashion the number of fertile males, number of inseminated females, number of litters delivered, testosterone levels, epididymal sperm count, and motility (Bajaj and Gupta 2012). These discrepancies among studies may come into view because of different environmental features such as animal species, cactus vegetative stage, period and quantity of supplementation, production system, management practices or even a poor rangeland condition, among others. Besides, although in some females exposed to the male effect, the induced ovulation is followed by an abnormally short luteal phase generating a "short cycle" due to a loss of thecal expression of the steroid acute regulatory protein (STAR), in our study, priming with progesterone to the experimental units banned the presence of short cycles (Chemineau et al. 2006; Alvarado-Espino et al. 2016).

Results from our study suggest that the male effect was able to successfully invoke neurophysiological pathways reactivating ovarian follicular and luteal cascades during the natural anestrous season. Yet, such physiological success was not equally exerted to promote an amplified embryo implantation percentage. Interestingly, a previous work of our group demonstrated that high-protein supplemented ewes prior to breeding depicted not only the lowest fertility rate, but also the highest embryonic mortality along with the lowest uterine pH (Meza-Herrera et al. 2006).

Later on, it was established that peri-conceptional high-protein supplementation in adult ewes besides to generate the lowest uterine pH; it also promoted the smallest conceptus weight, while also tended to secrete less INF-τ and IGF-1, whereas the correspondent endometrial explants depicted a higher basal $PGF_{2\alpha}$ release, all together compromising the establishment of an endometrial milieu prone to the maternal recognition of pregnancy process (Meza-Herrera et al. 2010; Meza-Herrera and Tena-Sempere 2012). Such physiological scenario may has been involved in the decreased response observed in our study regarding the reduced embryonic implantation rate, at least in the PEO goats. Admittedly, however, this tempting possible physiological scenario is yet to be experimentally proven.

Based on our results, there is an essential need to align not only the peri-conceptional but also the peri-implantation stages to the best suited environmental conditions in the rangeland. Preliminary observations at field level indicate that goat producers are prone to the adoption of this technological package. The adoption of both management strategies targeted *Opuntia* supplementation, and the male effect should support increases in both reproductive and economic efficiency while promoting sustainability in those rangeland-based marginal goat production systems.

Conclusions

No differences were observed among treatments for any of the studied ovarian and reproductive response variables. Yet, the stimulus of the male effect successfully invoked re-activation of ovarian follicular and luteal pathways during the natural anestrous season in the female goat. Nevertheless, such successful physiological scenario was not equally exerted to promote an increased embryo implantation rate; this issue claims further consideration.

Acknowledgments This study was carried out within the outlined actions of the International Collaborative Projects "CONACYT-FOMIX-DURANGO, DGO-2008-C01-87559, and CONACYT-DGO-2009-C02-116746," funded by the National Council of Science and Technology, Mexico, as well as ALFA-III-ALAS, DCI-ALA/A9.09.01/08/19189/161-358/ALFA-III-82, supported by the European Union as well as by the UACH-DGIP, Mexico. *In loving memory*, *Georgina Robles-Orozco* (*1963-2015*).

Compliance with ethical standards

Conflict of interests None.

References

Akanni G.B., du Preez J.C., Steyn L., Kilian S.G. 2015. Protein enrichment of an *Opuntia ficus-indica* cladode hydrolysate by cultivation of *Candida utilis* and *Kluyveromyces marxianus*. Journal of Science of Food Agriculture, 95, 1094–1102.

Alvarado-Espino A.S., Meza-Herrera C.A., Carrillo E., González-Álvarez V.H., Guillen-Muñoz J.M., Ángel-García O., Mellado M., Véliz-Deras F.G. 2016. Reproductive outcomes of Alpine goats primed with progesterone and treated with human chorionic gonadotropin during the anestrus-to-estrus transition season. Animal Reproduction Science, 167, 133–138

Bajaj V.K., Gupta R.S. 2012. Fertility suppression in male albino rats by administration of methanolic extract of *Opuntia dillenii*. Andrologia, 44, Suppl 1, 530–537.

Chemineau P., Pellicer-Rubio M.T., Lassoued N., Khaldi G., Monniaux D. 2006. Male-induced short oestrus and ovarian cycles in sheep and goats: a working hypothesis. Reproduction Nutrition Development, 46, 417–429.

Díaz-Plascencia D., Rodríguez-Muela C., Mancillas-Flores P., Ruíz-Holguín N., Mena-Mungía S., Salvador-Torres F., Duran-Melendez L. 2012. In vitro fermentation of forage *Opuntia* inoculated with *Kluyveromyces lactis* obtained from apple residues. Revista Electronica de Veterinaria, 13, 1–11.

Dickie A., Paterson M.M., Anderson C., Boyd J.S. 1999. Determination of corpora lutea numbers in Booroola-Texel ewes using transrectal ultrasound. Theriogenology, 51, 1209–1224.

El-Mostafa K., El Kharrassi Y., Badreddine A., Andreoletti P., Vamecq J., El Kebbaj M.S, Latruffe N., Lizard G., Nasser B., Cherkaoui-Malki M. 2014. Nopal cactus (*Opuntia ficus-indica*) as a source of bioactive compounds for nutrition, health and disease. Molecules, 19, 14879–14901.

FASS 2010. Guide for the care and use of agricultural animals in agricultural research and teaching. 3rd Edition, Federation Animal Science Society, Champaign, **177 p**.

Flores-Najera M.J., Meza-Herrera, C,A., Echavarria, F.G., Villagomez, E., Iñiguez, L., Salinas, H., Gonzalez-Bulnes, A. 2010. Influence of nutritional and socio-sexual cues upon reproductive efficiency of goats exposed to the male effect under extensive conditions. Animal Production Science. 50, 897–901.

Gelez H., Fabre-Nys C. 2004. "The male effect" in sheep and goats: a review of respective roles of the two olfactory system. Hormones and Behavior, 6, 257–271.

Gonzalez-Bulnes A., Meza-Herrera C.A., Rekik M., H. Ben Salem, Kridli R.T. 2011. Limiting factors and strategies for improving reproductive outputs of small ruminants reared in semi-arid environments, Chapter 2. In: Semi-arid environments: agriculture, water supply and vegetation. Ed: K.M. Degenovine. Nova Science Publishers Inc. Hauppauge., p. 41–60.

Luna-Orozco J.R., Guillen-Muñoz J.M., De Santiago-Miramontes M.A., Garcia J.E., Rodriguez-Martinez R., Meza-Herrera C.A., Mellado M., Veliz F.G. 2012. Influence of sexually inactive bucks subjected to long photoperiod or testosterone on induction of estrus in anovulatory goats. Tropical Animal Health & Production, 44, 71–75.

Martin G.B., Oldham C.M., Cognie Y., Pearce D.T. 1986. The physiological responses of anovulatory ewes to the introduction of rams—a review. Livestock Production Science, 15, 219–247.

Mellado, M., Rodriguez, A., Lozano, E.A., Dueñez, J., Aguilar, C.N., Arevalo, J.R. 2012. The food habits of goats on rangelands with different amounts of fourwing saltbush (*Atriplex canecens*) cover. J. Arid Environ. 84:91–96.

Meza-Herrera C.A., Tena-Sempere M. 2012. Interface between nutrition and reproduction: the very basis of production. In: eds. S. Astiz, A. Gonzalez-Bulnes Animal reproduction in livestock, in Encyclopedia of Life Support Systems (EOLSS), under the auspices of the UNESCO, Eolss Publishers, Oxford, **[http://www.eolss.net]**.

Meza-Herrera, C.A., Ross T., Hawkins D., Hallford D. 2006. Interactions between metabolic status, pre-breeding protein supplementation, uterine pH, and embryonic mortality in ewes: preliminary observations. Tropical Animal Health & Production, 38, 407–413.

Meza-Herrera C.A., Ross T., Hallford D, Hawkins D., Gonzalez-Bulnes A. 2010. High periconceptional protein intake modifies uterine and embryonic relationships increasing early pregnancy losses and embryo growth retardation in sheep. Reproduction in Domestic Animals, 45, 723–728.

Mullins K.A., Matthews H.H., Griffin W.M., Anex R. 2014. Impacts of variability in cellulosic biomass yields on energy security. Environmental Science & Technology, 48, 7215–7221.

NAM—National Academy of Medicine. 2002. Guide for the care and use of laboratory animals. Co-produced by the National Academy of Medicine—Mexico and the Association for Assessment and Accreditation of Laboratory Animal Care International. 1st ed. Harlan, Mexico.

NRC (2007): Nutrient requirements of small ruminants: Sheep, goats, cervids and new world camelids. National Academy Press, Washington, DC, USA.

Rekik M., J. Ben Salem, Lasoued N., Chalouati H., I. Ben Salem 2010. Supplementation of Barbarine ewes with spineless cactus (*Opuntia ficus-indica F. inermis*) cladodes during late gestation-early suckling: effects on mammary secretions, blood metabolites, lamb growth and postpartum ovarian activity. Small Ruminant Research, 90, 53–57.

Rekik M., Gonzalez-Bulnes A., Lassoued N., H. Ben Salem, Tounsi A., I. Ben Salem 2012. The cactus effect: an alternative to the lupin effect for increasing ovulation rate in sheep reared in semi-arid regions? Journal of Animal Physiology & Animal Nutrition, 96, 242–249.

Ren J., Tan S., Dong L., Mazzi A., Scipioni A., Sovacool B.K. 2014. Determining the life cycle energy efficiency of six biofuel systems in China: a Data Envelopment Analysis Bioresource Technology, 162, 1–7.

Sakly C., Rekik M., Ben Salem I., Lassoued N., Mtaallah B., Kraiem K., Gonzalez-Bulnes A. 2012. Reproductive response of Barbarine ewes to supplementation with alternative feed prior to and during mating under semi-arid extensive conditions. European Association of Animal Production, 131, 235–239.

Sakly C., Rekik M., I. Ben Salem, Lassoued L., Gonzalez-Bulnes A., H. Ben Salem 2014. Reproductive response of fat-tailed Barbarine ewes subjected to short-term nutritional treatments including spineless cactus (*Opuntia ficus-indica F. inermis*) cladodes. Journal of Animal Physiology & Animal Nutrition, 98, 43–49.

Urrutia-Morales J., Meza-Herrera C.A., Escobar-Medina F.J., Gamez-Vazquez H.G., Ramirez-Andrade B.M., Diaz-Gomez M.O., Gonzalez-Bulnes A. 2009. Relative roles of photoperiodic and nutritional cues in modulating ovarian activity in goats. Reproductive Biology, 9, 283–294.