academicJournals

Vol. 14(9), pp. 719-727, 4 March, 2015
DOI: 10.5897/AJB2014.14300
Article Number: 7FCD94450949
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Review

Status and implementation of reproductive technologies in goats in emerging countries

Aime J. Garza Arredondo[1], Adan González Gómez[2], José Fernando Vázquez-Armijo[3], Rogelio Alejandro Ledezma-Torres[1], Hugo Bernal-Barragán[2] and Fernando Sánchez-Dávila[2]*

[1]Universidad Autónoma de Nuevo León, Facultad de Medicina Veterinaria y Zootecnia.
[2]Universidad Autónoma de Nuevo León, Facultad de Agronomía, México.
[3]Centro Universitario UAEM Temascaltepec, Universidad Autónoma del Estado de México.

Received 3 November, 2014; Accepted 19 February, 2015

In modern cattle breeding, assisted reproductive technologies in small ruminants have been used even out-of-season breeding, but in emerging countries these technologies have been mainly used for estrous synchronization and artificial insemination. However, in the last 30 years, significant progress in the matter of transfers of *in vivo* derived and *in vitro* produced embryos has been achieved. Currently, eight donor embryos are obtained by goat, due to the physiological knowledge of the presence of dominant follicles; above alter results recovered embryos (4 embryos / donor). Whereas, the goat has the advantage of presenting a shorter interval between generations compared to cow; this promote more research in *in vitro* fertilization, and overcome the limitations presented in a traditional embryo transfer. However cloning and transgenesis, are two techniques that will begin to have a commercial application, because goats can be used as bioreactors to produce milk with specific proteins to develop drugs. The objective of this study was to establish and describe in a general way the use of these techniques in goats, to promote their use so as to achieve genetic improvement in goats.

Key words: Reproductive technologies, goats, estrous synchronization, embryo transfer.

INTRODUCTION

Worldwide, goat population is predominantly distributed in tropical, subtropical and semi-desert rural areas under unfavorable nutritional conditions (Holtz, 2005; Mellado, 2008). Likewise, goats are characterized as being one of the most fertile domestic species, with up to 90% conception rate and the litter size varies from 1 to 1.5 offspring each year, depending on the breed, season and environmental conditions where it is exploited (Mellado, 2008; Cseh et al., 2012). On the other hand, the natural breeding season of breeds exploited in temperate areas is restricted to the beginning of autumn and winter, so mother goats give birth in early spring, which coincides with natural growth of vegetation (Delgadillo et al., 1999; Jackson et al., 2006). Since goats belong to the group of species subject to seasonal fluctuations and considering the different geographical areas in which they are exploited, their limited genetic improvement blends with the photoperiod effect (Delgadillo et al., 1999), the

*Corresponding author. E-mail: fernando_sd3@hotmail.com.

nutritional effect (Scaramuzzi et al., 2006) and the health effect (Cseh et al., 2012), as well as sociocultural aspects of the majority of the regions in which goats are exploited and that almost 90% of goat producers have little knowledge of new technologies (Mellado, 2008). Taking into consideration the aforementioned, the application of new technologies in small ruminants has been restricted in developing countries (Chang et al., 2006; Lehloenya and Greyling, 2010; Paramio, 2010; Gama and Bressan, 2011; Guerra et al., 2011).

Traditionally, transfer of *in vivo* produced embryos include: estrous synchronization techniques, artificial insemination, superovulation treatments and embryo implantation (Sánchez-Dávila et al., 2014). However, in countries with large goat population, the high costs of this methodology have restricted the use of these techniques in a massive way. An alternative could be to work with embryos produced *in vitro*, in order to be offered cheaper and shorten the generation interval used in this technique compared to the *in vivo* traditional technique. Since in a traditional MOET programme, the interval between generations is approximately one year, through *in vitro* produced embryos, this can be substantially reduced by half (5 to 6 months) and theoretically, can double the rate of genetic gain in the herd (Magalhaes et al., 2011; Amiridis and Cseh, 2012; Baldassarre, 2012). Additionally, genetic gain can be improved using sex-sorted semen; however, the use of this technology is very restricted in small ruminants compared to bovines. According to the International Embryo Transfer Society, in 2009, the number of embryos produced *in vivo* or *in vitro* for bovines and small ruminants were 842 376 and 2 086, respectively (Cseh et al., 2012).

Therefore, the objective of the present study was to describe in a general way the restrictions and advantages of using assisted reproductive technologies in goats and worldwide current trends.

ARTIFICIAL INSEMINATION

Great part of the success of genetic improvement in goats worldwide has been the use of artificial insemination (AI) in programmes for the upgrading of goats (Ahmed et al., 2011), since this improvement is achieved by using an “improved” buck, it requires the presence of great number of superior bucks to be evaluated, selected and finally, their genetic value must be compared with reference males (Gama and Bressan, 2011; Cseh et al., 2012). For instance, in countries such as France, the systematic use of AI for genetic improvement of dairy goats, is currently part of the routine management of the exploitation. The AI technique in goats could have additional advantages by using it massively, since goat herds are geographically dispersed and they are a species subject to seasonal fluctuations. Basically, as AI technique itself, three techniques have been promoted which are already in use worldwide (Holtz, 2005; Amiridis and Cseh, 2012).

The first technique is transcervical insemination, a method in which the semen is transferred into the uterus, using a rigid endoscope, a light source and an insemination gun with recommended dosage between 80 and 100 million of spermatozoids (Holtz, 2005). When artificial insemination is performed using fresh, diluted semen, conception rates can be comparable to those in natural breeding (Paramio, 2010). Conception rates of minimum 50% and as good average, 60 to 65% are reported worldwide. However, some breeds have only reached 40% (Angora breed) (Tasmedir et al., 2011). In Mexico, the extensive use of AI has been concentrated in two states: Guanajuato and Coahuila, due to governmental programmes since 2000. The results obtained in these states show an annual positive genetic trend for milk production of 2.99 ± 1.06 kg (0.32%), still far below the 1 to 2% obtained in France (Torres-Vásquez et al., 2010). In Brazil, pregnancy rates of 59% by transcervical route are reported, using fresh, diluted semen at 5°C and coconut water as diluent, mainly in dairy breeds, such as Saanen. As in other countries, different protocols for estrous synchronization have been used in Brazil, such as the use of devices between 5 and seven days, reaching pregnancy rates between 60 and 73% (Guerra et al., 2011).

The second technique for AI in goats is laparoscopy, which was initially used in the 80s in sheep by a group of Australian researchers; currently, countries in South America, mainly Argentine, Brazil and Uruguay, have obtained successful results since the 90s. In general, substantial and consistent pregnancy rates have been achieved with the use of this technique in comparison with the transcervical method (Holtz, 2005). This technique requires the use of specialized equipment, which includes: a light source, optical fibre, and dedicated pipettes from global enterprises. The doe is fasted with no access to water for a minimum of 12 h before the procedure is performed. The goat is then placed in a laparoscopy cradle, positioned in a supine head-down position to an approximate angle of 45° (Cseh et al., 2012). Subsequently, a small skin incision is made 10 cm ventral to the udder on each side of the midline for introducing laparoscope and insemination pipette. A light stab action is made and one-half of the semen dose is deposited in each uterine horn at approximately 5 cm from the uterine bifurcation. In emerging countries, the use of these techniques has been directed towards evaluating the variations in time periods of AI, semen dose, sperm concentration, type of semen used (fresh, diluted, refrigerated or frozen-thawed semen), finding variable pregnancy rates ranging from 43 to 68% (Gama and Bressan, 2011; Guerra et al., 2011).

The third technique developed in Germany is to expose the cervix and deposit semen in the uterine horns by

transcervical route, reporting up to 71% of births compared to 51% obtained by laparoscopy (Holtz, 2005). The use of this technique has been restricted because no study has been reported. Future research should be conducted to standardize protocols for synchronization of ovulation to achieve pregnancy rates increase using IATF; and intensify research regarding male effect and achieve synchrony of estrous and inseminated at estrous observed. This is with the aim of reducing the use of hormones and produce meat and milk are free of hormones and are labeled as ethical and green.

Estrous cycle control (estrous synchronization)

In a simplified form, the aim of estrous synchronization is that a group of goats show a fertile estrous within a specified time period between an average of 24 and 48 h and be able to carry out breeding services, either natural breeding or AI, using one of the aforementioned techniques (Holtz, 2005; Fonseca et al., 2005; Fatet et al., 2011; Cseh et al., 2012; Amiridis and Cseh, 2012; Álvarez et al., 2013). At global level, estrous synchronization protocols developed in the major centers for goat (France) and sheep production (Australia), to later disseminate worldwide; following an important growth in Uruguay, Brazil, Argentine and Mexico, speeding up this process by beginning to use real-time ultrasonography, in order to understand and explain the follicular dynamics in small ruminants, mainly in sheep and goats (Leboeuf et al., 2000; Mellado, 2008; Menchaca et al., 2010; Guerra et al., 2011).

As with the majority of countries worldwide, the protocols used are based on the use of progestogen or progesterone treatment in the form of vaginal devices (sponges/CIDR) or ear implants (Fonseca et al., 2005; Abecia et al., 2012; Vilariño et al., 2011). This hormonal manipulation can be used either out or during breeding season, by means of estrous synchronization protocols, which mainly differ in the type of device to be used, its duration, the combination of prostaglandin analogues, as well as the moment of application of hormones that induce and synchronize ovulation, such as: equine chorionic gonadotrophin (eCG), estradiol benzoate and GnRH (Menchaca and Rubianes, 2007; Menchaca et al., 2009; Modu-Bukar et al., 2012). However, the use of progestogen may cause several responses depending on the cycle phase in which treatment is initiated; for instance, during the luteal phase, follicular development speeds up, but at the same time, the number of large follicles decreases, increasing the rate of follicular atresia and supporting the persistence of large estrogenic follicles (Nava et al., 2010). During the follicular phase, the number of large follicles and ovulation rate decreases (Riaz et al., 2012).

However, the use of progestogen sources during the period of sexual rest induces a form of diestrous that generates normal ovarian follicle development. When the progestogen is removed, the follicles can ovulate during the season in which breeding fails, due to seasonal negative feedback action of the hormone, thus it is necessary that the gonadotropin stimulates total follicle maturation and ovulation. In each case, the use of vaginal devices increases progesterone concentration, but on the second day, it starts to decline, which physiologically alters Luteinizing Hormone (LH) secretion. Several alternatives were developed to improve the aforementioned; one of them is the substitution of the vaginal device at day seven, in comparison with the synchronization programme which traditionally substitutes the device between days 11 and 14 (Baldassarre, 2012) and also a prostaglandin F2α ($PGF_{2\alpha}$) dose is administered at the moment that the second device is removed. A second option is the use of short protocols (five to seven days) with vaginal devices, which began to develop in the 90s with satisfactory results with respect to the rate of synchronization and ovulation, either in goats or in sheep (Menchaca et al., 2007; Menchaca et al., 2010). It has been determined that short estrous synchronization protocol induces LH peak from 40 h and ovulation takes place 60 h after the completion of the progesterone treatment (Menchaca et al., 2007). When using short estrous synchronization protocols, the goats show a new wave of follicles 3.5 days after the insertion of the device, allowing that in embryo transfer programmes, the development of dominant follicles is avoided and better embryonic response will be obtained (Fonseca et al., 2013). Currently, the use of recycled CIDR has played a key role in the projection of similar results between new CIDR (62.5%), sterilized CIDR (79.5%) and CIDR placed in autoclaves (69%) with respect to pregnancy rate and estrous rate from 97.5 to 100% (Álvarez et al., 2013). Researchers in South America confirm that the use of recycled CIDR does not affect the rate of fertility and pregnancy (Vilariño et al., 2011; Oliviera et al., 2013). Another device which has been used in sheep and goat has been the implant impregnated with the potent progestogen norgestomet (Crestar), which is inserted in the middle part of the animal's ear and one-half of the implant is usually used in goats. The use of CIDR and subcutaneous implants in goats showing poor physical development and in goats kidding for the first time, may be more comfortable in comparison to sponges that cause stress in the animals and they are very difficult to remove (Holtz, 2005; Palvenz et al., 2005; Souza et al., 2011; Penna et al., 2013).

Accordingly, in the last decade, these two aspects have been modified in a vast body of scientific literature worldwide; however, the limitations of the use of synchronization protocols, short or long, because of vaginitis problems and presence of progesterone in milk, are justified by a pregnancy rate of 60% (López-Sebastián et

al., 2007; Menchaca et al., 2007). Nevertheless, they have gained strength in the European Community and the marketing of this milk is still legal in our country on a large scale (Mellado, 2008). An interesting point, linked to the previous, is the use of eCG in the synchronization protocols, where the best pregnancy results are obtained (61%), compared to other sources of synchronization of ovulation, such as: estradiol benzoate (40%) and GnRH (39%) (Menchaca et al., 2007; Zarazaga et al., 2014) considering that further studies must be done to evaluate alternative sources of ovulation, as previously described. Although, it has been reported that successive use of eCG generates development of antibodies, it is still used in countries such as Mexico and South America, where pregnancy rate has not been evaluated, due to repeated use of eCG in goat herds. Other synchronization protocols have been use based on the use of prostaglandin F2α analogues (cloprostenol, dinoprost tromethamine), which are potent luteolytic agents and can be used during breeding season as an alternative for estrous synchronization. It has been reported that PGF2α effectivity is limited between day 3 and 14 of the estrous cycle and affects the time of LH peak and subsequent ovulation (Holtz, 2005). Another way to use PGF2α is to combine it with the male effect and a source of progesterone (López-Sebastián et al., 2007), where the treatment involves administering 25 mg of progesterone in olive oil by intramuscular route at the moment of introducing the male, followed by a dose of 75 µg of cloprostenol by intramuscular route, seven days after exposing the male to the females. Based on this synchronization protocol, a pregnancy rate of 65% is achieved, compared to the traditional protocols of vaginal device and the use of eCG at a dose of 350 IU (47%). Other protocols that have been used in bovines, such as the ovsynch protocol, which is based on the administration of two doses of GnRH at intervals of 9 days and PGF2α is applied two days before the second administration of GnRH; this protocol is based on the fact that the first GnRH injection in the presence of a large follicle triggers ovulation and the formation of corpus luteum, considering that the administration of PGF2α will destroy both, the natural developed corpus luteum and the one developed by the first GnRH injection, so that the second GnRH injection triggers ovulation (Holtz et al., 2008). With the previous protocol, great synchronization of preovulatory LH peak can be achieved and pregnancy rates of 58% are obtained (Holtz et al., 2008).

Other natural techniques or so called sustainable are those related to the combined use of photoperiod, male effect and nutritional supplementation, for establishing alternative strategies and breeding management to the use of drugs (hormones) previously described. Although, the male effect has been known in sheep and in goats, groups of researchers are working strongly to be able to use the male effect in a more efficient way without the need of hormones. For instance, in sheep and in goats, the presence of short cycles is not uncommon when a male is introduced to a group of anoestrus females; however, a way to avoid it is to administer a dose of progesterone at the moment the male is introduced. Nevertheless, this methodology contrasts the use of hormonal products with the use of clean, green and ethical methods promoted by groups of researchers (Delgadillo et al., 2012). Since the 90's, the interest to determine the photoperiod is determinant for at least in some regions of seasonal anoestrus in goats and low libido in bucks (Pellicer-Rubio et al., 2008; Delgadillo et al., 2012), has led to use strategies for activating the male through light programmes that regulate the photoperiod of males, in such a way that they must be activated in the anoestrus season and be able of inducing estrous in the anoestrus goat (Delgadillo et al., 2012), as well as the use of females in heat (Rodríguez-Martínez et al., 2013) or males treated with testosterone (Véliz et al., 2013). However, it has been demonstrated that as unique effect it can be affected by the male itself, varying or affecting age, breed, physical condition, forms of grouping, experience of itself, as well as hierarchy. On the other hand, the doe plays an important role in its response at the moment the buck is introduced, behaving differently according to the stage of anoestrus and breeding season, whereas during breeding season the effect could be minor, due to high progesterone levels and during the anoestrus season, the goat gets excited as soon as the male is introduced and the effect shows up because of an increase in LH (Delgadillo et al., 2012). In the breeding season, the male effect has better LH release output patterns when the goats are in the early and late luteal phase and not in the middle luteal phase; whereas high levels of progesterone inhibit further output of LH (Martínez-Álvarez et al., 2007).

On the other hand, previous isolation of the male is conditioned even in goats, which is currently determined that this is not a necessary thing to do, because being separated from the male or remaining in contact makes no difference in goats (Delgadillo et al., 2012). The male effect may increase breeding efficiency in goats and the behavioural signs of estrous increase in goats exposed to bucks (Prado et al., 2003). For instance, when goats are artificially inseminated, sterile service by using a vasectomized buck may reduce the time duration of estrous, increasing the rate of conception. The presence of the buck after removing the progestin or after the second injection of prostaglandin in synchronized goats reduces the time of pessary withdrawal or injection of prostaglandin for initiating estrous.

The male effect incorporates each aspect of a clean, green and ethical animal production. It does not need the use of exogenous hormones, has no negative effect in the environment and optimizes animal welfare; additionally, animals do not need to be handled or manipulated

at some moment to previous breeding. However, there are male effect limitations that restrict its deve-lopment as a reliable, repeatable and flexible tool that can be used in different breeds, seasons and latitudes. Consequently, it is proposed that the challenge for today's scientists is not only to find solutions to problems, but also understand when and why they happen. Future research should be directed to combine the use of hormones and the male effect. It requires conducting research that achieve standardized protocols synchroni-zation of ovulation. Intensify the use of short estrous synchronization protocols to make them more econo-mical, less time use of hormones and equal or better results than long estrous synchronization protocols.

Semen processing

In comparison with bovines, semen processing and its market in emerging countries, has been restricted, among other reasons, due to lack of adequate knowledge about the use of the insemination technique, where goat producers prefer to have higher pregnancy rates by natural breeding, instead of using goat semen genetically evaluated (Mara et al., 2007) whereas currently, purebred seed stock producers still rely on the importation of semen from European countries (France, Spain) and from the United States of America and Canada. Today, studies are focused on trying to improve the results of mass motility, progressive motility, abnormalities, live sperms, acrosomal disruption, among other goat semen variables (Mellado et al., 2006; Mainga et al., 2010). Now, studies have been conducted to efficiently use the egg yolk (Salmani et al., 2014) under different concentrations, used as an antioxidant (Ahmed et al., 2011) and alternative use of non-animal origin sources (soy milk) (Khalifa and El-Saidy, 2006; Salmani et al., 2014). Alternatively, different times and temperatures of semen storage have been evaluated (Paulenz et al., 2005; Islam et al., 2006; Salvador et al., 2006; Nainga et al., 2010), as well as its wash and centrifugation for seminal plasma removal (Sariozkan et al., 2010). Likewise, it has been found that in the use of enzymes, such as arginine, an enzyme of the urea cycle, which has two isoforms of mammalian arginase (types I and II), type II plays an important role in the synthesis of polyamines and through the parameter of seminal plasma activity of arginase, there is a high correlation between sperm motility and sperm concentration (Turk et al., 2011). All the aforementioned has been carried out with the aim to avoid one of the biggest problems that goat semen has, such as presence of the coagulating enzyme of the egg yolk, which is secreted by bulbourethral glands and reacts upon contact with the egg yolk and hydrolyzes lecithin into fatty acids and spermicidal lysolecithin (Cseh et al., 2012). Likewise, the secretion of bulbourethral glands has a toxic reaction with milk; this effect has been identified as a lipase (glycoprotein), where this enzyme hydrolyzes trioline and triglycerides into free fatty acids, which decrease sperm motility and harm the sperm acrosomal membrane (Shia et al., 2010). The use of frozen semen overcomes farm dispersion problems, enhancing the more accurate genetic evaluation of animals. However, the success of AI with frozen–thawed semen is lower than with fresh or cooled semen or that achieved by natural service. In Alpine and Saanen breeds, Guerra et al. (2011) observed a kidding rate of 75 and 44% for fresh and frozen semen, respectively. Overall, AI fertility rates with frozen–thawed semen vary from 40 to 60%, and seem to be highly affected by factors such as season, farm (Ahmed et al., 2011; Cseh and Amiridis, 2012), reproductive status of female (Leboeuf et al., 2000), and site of semen deposition (Paulenz et al., 2005).

Transfer of *in vivo* and *in vitro* produced embryos

Embryo transfer (ET) consists in administering high doses of follicle-stimulating hormone (FSH) of porcine or ovine origin, with the aim to obtain maximum follicular growth, which develops to the preovulatory follicle stage (González-Bulnes et al., 2004; Baldassarre, 2012). According to classic protocols of superovulation, follicular population starts to modify between 12 and 24 h after the administration of the first FSH dose (Sánchez et al., 2013). These changes in follicular population are associated with an increase in the total number of large follicles or equal to 2 mm, which grow to 5 mm in diameter in 12 to 48 h, reaching the preovulatory stage at 48 to 60 h (González-Bulnes et al., 2004). Although, the follicular growth pattern varies according to the FSH formulation and the administration protocol, the ovulatory follicles obtained as result of different protocols of superovulation are characterized by being smaller than the ovulatory follicles of non-stimulated, natural cycles (with gonadotropins) (Driancourt et al., 1991; Baldassarre, 2012; Melican and Gavin, 2008). This decrease in the size of ovulatory follicles has also been observed during natural cycles in all sheep breeds considered prolific, such as: Romanov, Booroola Merino and Chios (Paramio, 2010).

The FSH presents a short half-life; consequently, its effect on growth and estradiol production is maintained during shorter periods of time, this is why it has to be frequently administered (González-Bulnes et al., 2004; Baldassarre, 2012). FSH-superovulated goats present lower incidence of premature regression of corpus luteum in comparison with other treatments, such as eCG, that also causes luteolysis, due to FSH effect on estradiol secretion. Therefore, after ovulation, its residual effect is low and its effect declines on the production of estradiol,

thus triggering, in smaller number of cases, the cascade of events that lead to prostaglandin F2α secretion (González-Bulnes et al., 2004). FSH superovulation protocols have indeed the disadvantage in that the hormone has to be frequently administered, due to the similarity of physiological secretion pattern of FSH during the follicular stage of the goat and the heterogeneous response of animals to treatment, mainly because of limiting factors, both extrinsic (origin, purity and protocol of gonadotropin administration) and intrinsic (breed, age, and nutritional and reproductive status), of the animal (Melican and Gavin, 2008; Menchaca et al., 2010). An average of six to eight transferable embryos per donor can be produced in a successful goat MOET program (Baril et al., 1993; Cognié, 1999; Cognié et al., 2003). These results, however, depend on many factors (including breed, age and nutrition) that contribute to the high variability. It is common for the number of transferable embryos to range from 0 to 30 per donor with 25 to 50% of the donors failing to produce any transferable embryos due to fertilization failure and early regression of corpora lutea (González-Bulnes et al., 2004; Lehloenya et al., 2008; Baldasarre, 2012). Multiple ovulation and embryo transfer in small ruminants in many countries are restricted to the breeding season, due to seasonal cyclic activity (Delgadillo et al., 2012). For instance, in South Africa, it has been reported that Boer goats show maximum sexual activity in autumn and minimum activity in spring (Lehloenya and Greyling, 2010). This coincides with González-Bulnes et al. (2004), who report that certain goat breeds manifest the highest ovulation rate and embryo performance during the breeding season and that the minimum ovulation rate, as well as embryo performance are recorded in the anoestrus period; however, Lehloenya et al. (2008) and Sánchez-Dávila et al. (2004), found that multiple ovulation is independent of the breeding and non-breeding season in Boer and dairy goats. However, currently, attempts have been made for changing the strategies of embryo collection, intensifying the attempts to avoid laparotomy and consequently, increase the reproductive life of donors with high genetic potential. For instance, Fonseca et al. (2013) have evaluated the nonsurgical technique along with short protocols of estrus synchronization, with promising results for applying this technique worldwide; where they found that the percentage of recovery by wash medium was higher than 90% and 13.4 ± 4.1 of embryos recovered per female donor.

On the other hand, *in vitro* embryo production has been developed a little bit more constantly in small ruminants, but more in sheep than in goats (Sánchez et al., 2013). This technology requires specific manipulations, such as: collection and maturation of oocytes, fertilization and development of zygotes, and embryos already developed are frozen or fresh transferred (Amirdis and Cseh, 2012).

However, still today, the fact that embryos produced *in vivo* are generally of higher quality than embryos produced *in vitro*, due to their high implantation rate and to better freezing tolerance (Baldassarre, 2012). Today, still research purposes, oocytes are routinely collected from slaughtered females. After removing their ovaries and observing the presence of certain size of follicles, oocytes are aspirated from each one of the follicles with sterile syringes. Conversely, it can be done in live animals by laparotomy, which is used less often, or by laparoscopy. This collection can be preceded by FSH or eCG administration for ovarian stimulation or by non-stimulated animals. It has been reported that aspiration of available follicles decreases in time, taking into consideration that gonadotropin dose, time of aspiration with respect to stimulation, aspiration pressure and the donor, are more determinant factors in cumulus-oocyte complex recovery rate. Likewise, it is mentioned that oocyte collection can be carried out both during breeding season and non-breeding season; considering that in the latter, melatonin implants can be used to improve the number of available follicles and oocyte development (Holtz, 2005; Baldassarre, 2012) then, comes maturation process (Amiridis and Cseh, 2012). There have been great changes in the development of oocytes by using temperatures of 38 to 39°C for 24 h in a humid atmosphere with 5% of CO_2; from then on, oocytes exhibit the first polar body and metaphase II stage is completed. The most commonly used maturation medium is TCM-199 supplemented with pyruvate, inactive serum and hormones (FSH and LH); what is more, if it is used in combination with estrogens and FSH, blastocyst formation rate is improved. Currently, the combination of growth hormone and ICF-I at a rate of one dose of 100 ng/ml in the maturation medium has been used for obtaining a more uniform maturation of oocytes. Likewise, the inclusion of follicular fluid from non-atrestic follicles or from goats treated with gonadotropin in the maturation medium, may have some beneficial effect on oocyte *in vitro* maturation, because the follicular fluid contains a number of peptides, steroids and growth factors (Paramio, 2010, Tasdemir et al., 2011; Amiridis and Cseh, 2012).

Oocytes with development capacity can be selected from ovaries of slaughtered goats. Between 1,5 and 2,1 oocytes per ovary are obtained by aspiration or dissection of follicles. Additionally, cumulus-oocyte complex (COC) recovery by cutting the ovary is a simple and more efficient technique in comparison with aspiration and perforation methods. Follicles larger than 3 mm have more cumulus layers and show a better *in vitro* maturation, circumstances to be taken into account for selecting oocytes at the end of the growth stage. Oocytes from large follicles (> 5 mm in diameter) generate greater number of blastocysts than medium-small follicles (< 5 mm in diameter). An average of 9 COC are obtained per

ovary after preparing the goats with FSH. Oocyte collection by aspiration under laparoscopic observation of anatomic structures of genetically superior goats, allows the recovery after FSH priming of 3-4 COC per ovary. The effects of medium supplementation using gluco-proteins (LH, FSH, hCG and TSH) during *in vitro* maturation of goat oocytes, show that these hormones are required for successful development of oocytes in AI.

TRANSGENESIS

This technique has been considered the great hope of the human being, because great quantities of catalytic proteins can be produced. These have great effect on certain human diseases, such as the human stimulating factor of granulocyte colonies. The objective of this technique is to manipulate the DNA of an individual so that it expresses its genetic potential and the production of proteins at large scale can be achieved, since they are of great benefit to human health and pharmaceutical industry. Unlike bovines, the goat is an excellent model for transgenesis worldwide (Paramio, 2014). Also, they are potentially ideal bioreactor animals for producing great quantities of recombinant proteins of pharmaceutical use (Guerra et al., 2011), taking into consideration that just as in bovines, there is great loss of embryos when using this technique, but the great advantage of goats is that there is a very low percentage of placental retention, respiratory/cardiovascular dysfunction, abnormal postnatal development and postnatal losses (quotation). Additionally, goats are an important source of recombinant protein production, reaching 1 to 5 g/litre of milk produced by transgenic animals. In herds of transgenic animals, the production rate reaches 300 kg of this purified product per year (quotation). The methodology of animal cloning employing the technique used in the creation of the first transgenic animal “Dolly”, is still inefficient, even more in bovines, where perinatal losses are high, reaching 63% in the first three months of age of calves created by the protocol of somatic cell nuclear transfer (quotation). Considering that today, only recombinant human antithrombin III, produced by the European Community, is commercially manufactured (quotation).

FINAL CONSIDERATIONS

With respect to first-generation technology, as the case of estrous and ovulation synchronization, and AI, instead of standardizing the protocols of both techniques, they should be focused on their intensive use to increase the number of offspring as product of AI, using genetically outstanding bucks. In relation to semen, it is necessary to conduct further studies in strengthening acrosomal integrity, as well as to begin to use sex-sorted semen and process it without egg yolk, with the aim to export it without having health problems. With regard to embryo transfer, further studies should be carried out to improve embryo collection by avoiding, blocking or eliminating presence of dominant follicles, as well as to improve and disseminate nonsurgical technologies for obtaining more embryos from female donors during their reproductive life; whereas the tendency is to eliminate laparotomy method. Likewise, *in vitro* fertilization should be developed in emerging countries to achieve greater short-term genetic advance. However, the use of transgenesis is still restricted in this species, mainly due to cost-benefit of production. Based on the fact that assisted reproductive technologies aforementioned, must reach the field level in goat production systems for improving production and reproductive performance of goat herds.

Conflict of interest

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

Special thanks to the Department of Agronomy, Universidad Autónoma de Nuevo León (UANL).

REFERENCES

Abecia JA, Forcada F, and González-Bulnes A (2012). Hormonal control of reproduction in small ruminants. Anim. Reprod. Sci. 130:173-179.

Ahmed M, Wahida H, Rosnina Y, Gohb YM, Ebrahimib M, Nadiac FM, Audreyc G (2011). Effect of butylated hydroxytoluene on cryopreservation of Boer goat semen in Tris egg yolk extender. Anim. Repr. Sci. 129:44-49.

Alvarez L, Gamboa D, Zarco L, Ungerfeld R (2013). Response to the buck effect in goats primed with CIDRs, previously used CIDRs, or previously used autoclaved CIDRs during the non-breeding season. Liv. Sci. 155:459-462.

Amiridis GS and Cseh S (2012). Assisted reproductive technologies in the reproductive management of small ruminants. Anim. Repr. Sci. 130:152-161.

Baldasarre H (2012). Practical aspects for implementing in vitro embryo production and cloning programs in sheep and goats. Anim. Reprod. 9:188-194.

Chang Z, Fan ML, Wu-Tan J (2006). Factors affecting superovulation and embryo transfer in Boer goats. Asian Austr. J. Anim. Sci. 19:341-346.

Cseh S, Faigl V, Amiridis GS (2012). Semen processing and artificial insemination in health management of small ruminants. Anim. Repr. Sci. 130:187-192.

Delgadillo JA, Cañedo GA, Chemineau P, Guillaume D, Malpaux B (1999). Evidence for an annual reproductive rhythm independent of food availability in male creole goats in subtropical northern Mexico. Theriogenology 52:727-737.

Delgadillo JA, Duarte G, Flores JA, Vielma J, Hernández H, Fitz-Rodriguez G, Bedos M, Graciela Fernández I, Muñoz-Gutiérrez M, Retana-Márquez MS, Keller M (2012). Control of the sexual activity of goats without exogenous hormones: use of photoperiod, male effect and nutrition. Trop. Subtrop. Agroecos. 15:15-27.

Driancourt MA, Webb R, Fry RC (1991). Does follicular dominance occur in ewes? J. Reprod. Fert. 93:63-70.

Fatet A, Pellicer-Rubio MA, Leboeuf B (2011). Reproductive cycle of goats. Anim. Reprod. Sci. 124:211-219.
Fonseca JF, Bruschi JH, Santos ICC, Viana JHM, Maagalhaes ACM (2005). Induction of estrus in non lactating dairy goats with diferente estrous synchrony protocols. Anim. Repr. Sci. 85:117-124.
Fonseca JF, Zambrini FN, Alvim GP, Peixoto MGD, Verneque RS, Viana JHM (2013). Embryo production and recovery in goats by non-surgical transcervical technique. Small Rum. Res. 111:96-99.
Gama LT, Bressan MC (2011). Biotechnology applications for the sustainable management of goat genetic resources. Small Rum. Res. 98:133-146.
Gonzalez-Bulnes A, Baird DT, Campbell BK, Cocero MJ, García-García RM, Inskeep EK, Lopez-Sebastian A, McNeilly AS, Santiago-Moreno J, Souza CJH, Veiga-Lopez A (2004). Multiple factors affecting the efficiency of multiple ovulation and embryo transfer in sheep and goats. Repr. Fert. and Dev. 16:421-435.
Guerra MMP, Silva SV, Batista AM, Coleto ZF, Silva ECB, Monteiro Jr. PLJ, Carneiro GF (2011). Goat reproductive biotechnology in Brazil. Small Rum. Res. 98:157-163.
Holtz W (2005). Recent developments in assited reproduction in goats. Small Rum. Res. 60:95-110.
Holtz W, Sohnrey B, Gerland M, Driancourt MA (2008). Ovsynch synchronization and fixed time insemination in goats. Theriogenology 69:785-797.
Islam R, Ahmed K, Deka BC (2006). Effect of holding and washing on the quality of goat semen. Small Rum. Res. 66:51-57.
Jackson DJ, Fletcher CM, Keisler DH, Whitley NC (2006). Effect of melengesterol acetate (MGA) treatment or temporary kid removal on reproductive efficiency in meat goats. Small Rum. Res. 66:253-257.
Khalifa TAA and El-Saidy BE (2006). Pellet-freezing of Damascus goat semen in a chemically defined extender. Anim. Repr. Sci. 93:303-315.
Konyali C, Tomas C, Blanch E, Gomez EA, Graham JK, Moce E (2013). Optimizing conditions for treating goat semen with cholesterol-loaded cyclodextrins prior to freezing to improve cryosurvival. Cryobiology 67:124-131.
Leboeuf B, Restall B, Salamon S (2000). Production and storage of goat semen for artificial insemination. Anim. Repr. Sci. 62:113-141.
Lehloenya KC, Greyling JP. (2010). The ovarian response and embryo recovery rate in Boer goat does following different superovulation protocols, during the breeding season, Small Rum. Res. 88:38-43.
Lehloenya KC, Greyling JPC, Grobler S (2008). Effect of season on the superovulatory response in Boer goat does. Small Rum. Res. 78:74-79.
López-Sebastian A, González-Bulnes A, Carrizosa JA, Urrutia B, Días-Delfa C, Santiago-Moreno J, Gómez-Brunet A (2007). New estrus synchronization and artificial insemination protocol for goats base on male exposure, progesterone and cloprostenol during the non-breeding season. Theriogenology 68:1081-1087.
Magalhaes DM, Duarte ABG, Araujo VR, Brito IR, Soares TG, Lima IMT, Lopes CAP, Campello CC, Rodrigues APR, Figueiredo JR (2011). In vitro production of a caprine embryo from a preantral follicle cultured in media supplemented with growth hormone. Theriogenology 75:182-188.
Mara L, Dattena M, Pilichi S, Sanna D, Branca A, Cappai P (2007). Effect of different diluents on goat semen fertility. Anim. Reprod. Sci. 102:152-157.
Martínez-Álvarez LE, Hernández-Cerón J, González-Padilla E, Perera-Marín G, Valencia J (2007). Serum LH peak and ovulation following synchronized estrus in goats. Small Rum. Res. 69:124-128.
Melican D and Gavin W (2008). Repeat superovulation, non-surgical embryo recovery, and surgical embryo transfer in transgenic dairy goats. Theriogenology 69: 197-203.
Mellado M (2008). Técnicas para el manejo reproductivo de las cabras en agostadero, Trop Subtrop Agroecos. 9:47-63.
Mellado M, Pastora F, Lopez R, Rios F (2006). Relation between semen quality and rangeland diets of mixed-breed male goats. J. of Arid Env. 66:727-737.
Menchaca A, Miller V, Salveraglio V, Rubianes, E (2007). Endocrine, luteal and follicular responses after the use of the short-term protocol to synchronize ovulation in goats. Anim. Repr. Sci. 102:76-87.
Menchaca A and Rubianes E (2007). Pregnancy rate obtained with short-term protocol for timed artificial insemination in goats. Repr. Dom. Anim. 42:590-593.
Menchaca A, Vilariño M, Crispo M, de Castro T, Rubianes E (2010).
Menchaca A, Vilariño M, Pinczak A, Kmaid S, Saldaña JM (2009). Progesterone treatment, FSH plus eCG, GnRH administration and Day 0 protocol for MOET programs in sheep. Theriogenology 72:477-483.
Modu-Bukar M, Yusoff R, Haron AW, Dhaliwal GK, Goriman-Khan MA, Ariff OM (2012). Estrus response and folicular development in Boer does synchronized with flugestone acetate and PGF2α or their combination with eCG or FSH. Trop. Anim. Health and Prod. 44:1505-1511.
Nainga SW, Wahida H, Mohd Azamc K, Rosninaa Y, Zukib AB, Kazhala S, Bukara MM, Theind M, Kyawe T, San MM (2010). Effect of sugars on characteristics of Boer goat semen after Cryopreservation. Anim. Repr. Sci. 122: 23-28.
Nava TH, Chango VJ, Finol PG, Torres RP, Carrillo FF, Maldonado SJ, Gil HL, Adamou A (2010). Efecto de la dosis de eCG sobre la inducción del celo en cabras mestizas luego de un tratamiento cortó con Medroxiprogesterona. Rev. Cient. FCV-LUZ 20:181-183.
New approaches to superovulation and embryo transfer in small ruminants. Repr. Fert. and Dev. 22:113-118.
Oliveira JK, Martins G, Esteves JV, Penna B, Hamond C, Fonseca JF, Rodrigues AL, Brandao FZ, Lilenbaum W (2013). Changes in the vaginal flora of goats following a short-term protocol of oestrus induction and synchronisation with intravaginal sponges as well as their antimicrobial sensivity. Small Rum. Res. 113:162-166.
Paramio MT (2010). *In vivo* and *in vitro* embryo production in goats. Small Rum. Res. 89:144-148.
Paramio, MT and Izquierdo, D (2014). Assisted reproduction technologies in goats. Small Rum. Res. 121:21-26.
Paulenz H, Soderquistc L, Adnøyd T, Soltund K, Sæthere PA, Fjellsøye KR, Andersen- Berg K (2005). Effect of cervical and vaginal insemination with liquid semen stored at room temperatura on fertility of goats. Anim. Repr. Sci. 86:109-117.
Pellicer-Rubio MT, Leboeuf B, Bernelas D, Forgerit Y, Pougnard JL, Bonné JL, Senty E, Breton S, Brun F, Chemineau P (2008). High fertility using artifitial insemination during deep anoestrus after induction and synchronisation of ovulatory activity by the “male effect” in lactating goats subjected to treatment with artificial long days and progestagens. Anim. Repr. Sci. 109:172-188.
Penna B, Libonati H, Director A, Sarzedas AC, Martins G, Brandao FZ, Fonseca J, Lilenbaum W (2013). Progestin-impregnated intravaginal sponges for estrus induction and synchronization influences on goats vaginal flora and antimicrobial susceptibility. Anim. Repr. Sci. 142:71-74.
Prado V, Orihuela A, Lozano S, Isabel Pérez-León I (2003). Effect on ejaculatory performance and semen parameters of sexually-satiated male goats (Capra hircus) after changing the stimulus female. Theriogenology. 60: 261–267.
Riaz H, Sattar A, Arshad MA, Ahmad N (2012). Effect of synchronization protocols and GnRH treatment on the reproductive performance in goats. Small Rum. Res. 104:151-155.
Rodriguez-Martínez R, Angel-García O, Guillen-Muñoz JM, Robles-Trillo PA, De Santiago-Miramontes M de los A, Meza-Herrera CA, Mellado M, Véliz FG (2013). Estrus induction in anestrus mixed breed goats using the “female-to female effect”. Trop. Anim. Health Prod. 45: 911-915.
Salmani H, Towhidi A, Zhandi M, Bahreini M, Mohsen Sharafi M (2014).
Sánchez F, Bernal H, del Bosque A, González A, Olivares E, Padilla G, Ledezma R (2013). Superovulation and embryo quality with pFSH in Katahdin hair sheep during breeding season. A. J. Agr. Res. 8:2977-2982.
Sánchez-Dávila F, Ledezma-Torres, RA, Padilla-Rivas G, del Bosque-González AS, González-Gómez A, Bernal-Barragán H (2014). Effect of three levels of pFSH on superovulation and embryo quality in goats during two breeding seasons in Northeastern Mexico. Reprod. Dom. Anim. 49:40-43.

In vitro assessment of soybean lecithin and egg yolk based diluents for cryopreservation of goat semen. Cryobiol. 68:276-280.
Salvador I, Yániz J, Viudes-de-Castro MP, Gómez EA, Silvestre MA (2006). Effect of solid storage on caprine semen conservation at 5°C. Theriogenology 66:974-981.
Sarıozkan S, Bucak MN, Tuncer PB, Tas¸demir U, Kinet H, Ulutas PA (2010). Effects of different extenders and centrifugation/washing on postthaw microscopic-oxidative stress parameters and fertilizing ability of Angora buck sperm. Theriogenology 73:316-323.
Scaramuzzi RJS, Bruce KC, Jeff AD, Nigel RK, Muhammad K, Minerva MG, Anongnart S (2006). A review of the effects of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanisms that regulate folliculogenesis and ovulation rate. Repr. Nutr. Dev. 46:339-354.
Shia L, Zhang Ch, Yuea W, Shi L, Zhua X, Lei F (2010). Short-term effect of dietary selenium-enriched yeast on semen parameters, antioxidant status and Se concentration in goat seminal plasma. Anim. Feed Sci. Tech. 157:104-108.
Souza JMG, Torres CAA, Maia ALRS, Brandao FZ, Brushi, JH, Viana JHM (2011). Autoclaved, previously used intravaginal progesterone devices induces estrus and ovulation in anestrus Toggenburg goats. Anim. Repr. Sci. 129:50-55.
Tasdemir U, Reha AA, Kaymaz M, Karakas K (2011). Ovarian response and embryo yield of Angora and Lilis goats given the day 0 protocol for superovulation in the non-breeding season. Trop. Anim. Health Prod. 43:1035-1038.
Torres–Vázquez, J.A., Valencia–Posadas, M., Héctor Castillo–Juárez, H., Montaldo, H.H. 2010. Tendencias genéticas y fenotípicas para características de producción y composición de la leche en cabras Saanen de México. Rev. mex. de cienc. pecuarias 1:337-348.
Turk G, Gur S, Mehmet-Kandemir F, Sonmez M (2011). Relationship between seminal plasma arginase activity and semen quality in Saanen bucks. Small Rum. Res. 97:83-87.
Véliz FG, Meza-Herrera CA, De Santiago-Miramontes MA, Arellano-Rodriguez G, Leyva C, Rivas-Muñoz R, Mellado M (2009). Effect of parity and progesterone priming on induction of reproductive function in Saanen goats by buck exposure. Liv. Sci. 125:261-265.
Vilariño M, Rubianes E, Menchaca A (2011). Re-use of intravaginal progesterone devices associated with the Short-term Protocol for timed artificial insemination in goats. Theriogenology. 75: 1195-1200.
Zarazaga LA, Gatica MC, Gallego-Calvo L, Celi I, Guzmán JL (2014). The timing of oestrus the provulatory LH surge and ovulation in Blanca Andaluza goats synchronised by intravaginal progestagen sponge treatment is modified by season but not by body condition score. Anim. Reprod. Sci. Article in Press.