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Review Article

Reproductive Technologies in Camelids

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Abstract

Reproductive technologies have been developed to improve reproductive efficiency and genetics of animals as well as for infertility treatment. Great progress has been made in most farm animals. The camelids are probably the last large domestic species to experience the benefit from these technologies.

Camelids possess unique reproductive traits. Ovulation is induced by copulation and reproductive performance is low. Camelid semen is highly viscous with low sperm concentration. The viscous seminal plasma is a major impediment to the development of semen cryopreservation and other reproductive technologies.

The present review discusses the achievements of various biotechnological tools for reproduction [also known as assisted reproductive techniques (ARTs)] including the hormonal control of oestrus and ovulation, cryopreservation of gametes and embryos (collection and preservation of sperm, oocytes and embryos), artificial insemination (AI), in vitro fertilization (IVF), embryo transfer (ET) and reproductive cloning and emerging technologies. This paper aims to review developments and indicate which of these reproductive technologies can be used in the camel.

Keywords: Camelids, Semen, Reproductive technologies.

Introduction

Reproductive biotechnologies intend to be used routinely to shorten generational intervals and to propagate genetic material among breeding animal populations. To achieve this goal, reproductive technologies [also known as assisted reproductive techniques (ARTS)], have been developed

in generations over the years; namely artificial insemination (AI), embryo transfer (ET), manipulation of fertilization and embryo production in vitro (IVF/IVP) and multiplication techniques (cloning) for the application of transgenesis (Skidmore, 2003; Tibary et al., 2007; Anouassi and Tibary, 2013; Hemeida, 2013; Nagy et al., 2013; Skidmore et al., 2010, 2013; Waheed et al., 2014) . These, together with sperm separation techniques (Morrell and Rodriguez-Martinez, 2009, 2011), including that of selection of spermatozoa for chromosomal sex (commonly named sex-sorting), all face today a strong wave of increasing commercialization (Faber et al., 2003; Garner and Seidel, 2008; Seidel, 2009).

Use of most reproductive technologies; whether new or older, usually entails some costs. Costs may include time spent learning new procedures; and costs of supplies and services. Most new reproductive technologies are somewhat expensive, and some only make sense as research tools or for application in niche situations. Delivering the newer reproductive technologies usually is via older technologies, such as artificial insemination (AI) and embryo transfer(ET).

The present review discusses the achievements of various biotechnological tools for reproduction in live stock including semen handling for artificial insemination (AI), multiple ovulation and embryo transfer (MOET), in vitro handling of oocytes and production of embryos (IVF/IVP), reproductive cloning and emerging technologies (sex selection, gene targeting and nuclear transfer for livestock transgenesis, etc.). The application of these technologies for camelids breeding is critically discussed in relation to their impact in the improvement of the efficiency of their production and racing abilities.

Camelids

The Camelidae family comprises four domesticated species; the Bactrian camel (*Camelus bactrianus*), the dromedary *(Camelus dromedarius),* the llama (*Lama glama*) and alpaca (Vicugnapacos). All species have the same conservative karyotype (2n = 74) and can produce fertile hybrids between species; both within and even between genera (Skidmore et al., 1999; Wheeler et al., 2006).

For centuries, the camel has been a very important animal in the desert regions because of its ability to provide milk, meat, hair and transport in harsh, dry conditions. As camels are generally used in less well-developed countries, research into improving characteristics such as fertility and milk

and meat production have been lacking. However; the development of camel racing in the Middle East has led to an increase in value of the racing dromedary and thus increased interest in improving reproductive efficiency.

The low reproductive performance is one of the most important factors affecting camel productivity. Opportunities to improve reproductive efficiency in camels are limited by the advanced age at puberty, the long gestation period and the short breeding season, as well as the continuing use of traditional systems of reproductive management in most breeding herds (Skidmore, 2003; Skidmore and Billah, 2012). The use of assisted reproductive techniques for camel breeding has been slow to advance due to lack of detailed information on the reproductive physiology and to problems associated with the high viscosity and low sperm concentration in camelids ejaculate; and lack of established semen cryopreservation methods.

Semen Handling and Artificial Insemination

Genetic progress in most livestock can be increased up to 50% through the application of AI, the first generation biotechnology, using either extended semen that has been preserved in liquid form (fresh, or cooled to 5°C), or deep-frozen (Vishwanath, 2003). During the past 50 years, the development and application of cattle AI with preserved (either chilled or frozen) semen, using standardized methods for extension, cooling, freezing and thawing, have been growing exponentially on a global scale (Thibier and Wagner, 2000). Fertility after AI of fresh liquid-conserved semen is as good as natural mating (above 80%), while that of frozen semen is somewhat lower (yet usually above 60%). Improvements of the genetic basis of a herd take about 7–8 years when selected semen is used for AI (Rodriguez-Martinez, 2012).

The extensive and safe use of AI with preserved bull semen has led, incorporating the use of milk recording and of effective evaluation systems to the establishment of progeny testing systems for cattle (Norman et al., 2003). Inappropriate use of sires, selected without taking enough consideration to reproductive traits and focusing mainly for increased milk production led to a documented decline in reproduction success (Rodriguez-Martinez et al., 2008). Other associated disarrays, such as less intense oestrous signs leading to wrong timing for AI, have also been detected (Garcia et al., 2011).

Camelids, which include alpacas, llamas, vicunas; guanacos and dromedary and Bactrian camels, semen are characterized by a highly viscous, low-volume ejaculate with a low concentration of spermatozoa that exhibit low progressive motility (Hemeida et al., 2001; Skidmore et al., 2011; Kershaw-Young and Maxwell, 2012; Hemeida, 2013). The camelids viscous ejaculate, is believed to prevent sperm loss following mating and hold spermatozoa in the uterus until ovulation occurs, a favourable mechanism given to the relatively low sperm concentration in such ejaculate (Bravo et al., 2012; Kershaw-Young and Maxwell, 2011, 2012). The viscous seminal plasma hinders handling and dilution of semen and is currently the major impediment to the development of assisted reproductive technologies (ARTs).

As the biochemical components of camelid seminal plasma are somewhat similar to other domestic livestock species, except for low fructose and citric acid, semen extenders used for these species were tried for the storage of camelids semen. Semen extenders such as lactose-, sucrose-, citrate- and fructose-based buffers in addition to the commercially available extenders manufactured for other livestock species such as Green Buffer, Biladyl, Androhe, Triladyl, Bioxcell and INRA96 have been attempted with camelid semen. Best extenders for liquid storage of semen are: the Green Buffer plus 20% egg yolk (50% PR), Lactose-egg yolk extender (50% PR) or INRA extender (36%) (Bravo et al., 2000; Morton et al., 2008). Camels inseminated with fresh diluted semen (150-300 million sperm into uterine body) have yielded pregnancy rate of 40-50% (Bravo et al., 2000; Skidmore and Bellah, 2006). The post-thaw motility of frozen-thawed camelid spermatozoa averages 20% (Von Baer and Hellemann, 1999; Deen et al., 2003; Vaughan et al., 2003) and is rarely >40% (Bravo et al., 2000; Niasari-Naslaji et al., 2007). These post-thaw motility rates of camelid spermatozoa are unlikely to be commercially viable or adequate for AI. Pregnancy rates after AI with frozen-thawed semen in camelids are also low. In dromedary camels, only 1/13 camels were diagnosed pregnant following insemination with frozen-thawed spermatozoa (Deen et al., 2003), whereas in alpacas, no pregnancies were obtained using AI with frozen-thawed sperm (Vaughan et al., 2003). Post-thaw motility rates are higher (up to 40%) when the viscous seminal plasma is reduced by either mechanical (Niasari-Naslaji et al., 2007) or enzymatic (Bravo et al., 2000). When the viscosity of llama and alpaca semen was reduced, then cryopreserved used for AI of alpacas, 5 of 19

females (26%) gave birth to live cria (Bravo et al., 2000). Best liquefaction and progressive sperm motility is achieved in Tris-lactose egg yolk extender and Green Buffer for fresh semen of the dromedary camel (Wani et al., 2008; Morton et al., 2011).

An important step in camelid AI is the induction of ovulation in the females to be inseminated. Seminal plasma and GnRH were used to induce ovulation in artificially inseminated alpaca with 52% and 69% pregnancy rate, respectively (Huanca et al., 2012). GnRH induces ovulation in 26-32 hours, female camels inseminated with 150 million live sperm 24 hours after GnRH injection showed 53% pregnancy rate (Skidmore and Billah; 2006). Vasectomized males, GnRH and seminal plasma induced ovulation in alpaca with 72%, 76% and 77.6%, respectively and in llama with 91% and 100%, respectively (Bravo et al., 2012).

Embryo Transfer

The ET methodology is a suitable, more integrated approach for genetic distribution than AI, leading to improvement of genetic basis within 5 years (Rodriguez-Martinez, 2012). Moreover, as for AI, allows movement of material worldwide and reduces the risk of transmitting specific diseases. Although multiple ovulation and embryo transfer (MOET) would be considered advantageous as a methodology for genetic improvement, and up to 80% of embryos have been commercially transferred, the technology has not reached optimality because of the variability of the ovarian response to the superovulatory gonadotrophin treatment used so far (Mapletoft et al., 2002; Betteridge 2006; Lonergan, 2007). As a method, ET basically requires synchronization of the donor and the recipient females so that the embryos are recovered and transferred in synchrony (Rodriguez-Martinez et al., 1999). Embryo transfer does not have to be carried out immediately, and bovine embryos can be frozen, either conventionally (slow freezing using ethylene-glycol) or by nitrification (high concentrations of cryoprotectants and plunging into LN2), ensuring safe storage and better management of the genetic resources (Saragusty and Arav, 2011). Embryo transfer of in vivo (or in vitro) produced embryos to the uterus of a recipient cow is easy and reliably carried out by transcervical intrauterine deposition, with >60% of pregnancy rates. Use of transvaginal, ultrasound-guided follicular puncture for oocyte retrieval (commonly named OPU) may make MOET more effective because it waives superovulation and AI treatments; by the

collection of oocytes (up to 1000 oocytes can be collected from a heifer/cow per year) and following in vitro embryo production [up to 300 in vitro produced (IVP), embryos can be obtained per year] (Presicce et al., 2010).

In the camelid embryo transfer is a complex reproductive manipulation of female donor and surrogate camels involving a number of potentially limiting steps, any one of which can greatly influence the success of the procedure. Compared with other livestock, ET technique is more difficult in camels because they do not ovulate spontaneously and do not have a cyclic corpus luteum, these factors make superovulation of donors and preparation of the recipients less reliable. Under normal circumstances adult female camels have a gestation period averaging 13 months and as such only have a calf every two years. An embryo transfer program can allow a single donor female to produce up to 15 calves from multiple bulls in a single season (Tinson et al., 2012).

Regimens to synchronize estrus, and to induce ovulation and superovulatory responses have been developed in camelids. To control the follicle wave cycle; prostaglandin cannot be used in camel due to the lack of functional CL during reproductive cycles (Niasari-Naslaji, 2008). In addition, the beneficial application of progestogens is controversial in camelides. Several superovulation trials using follicle-stimulating hormone (FSH) from different species and equine chorionic gonadotropin (eCG) showed that the best time for initiation of superovulatory treatment is just after ovulation or when the ovaries do not present any follicles on ultrasonographic evaluation. Attempts to control the follicular wave with progesterone or progesterone and estradiol yielded variable results. Progesterone treatments for 7 to 12 days in combination with FSH or eCG have been used to induce superovulation in alpacas and llamas. In most cases, 2 to 6 ovulations are induced with a range of 0 to 12. Synchronization of female dromedaries with progesterone (PRID), for 12 days followed by an injection of 2000 iu eCG (im) improved reproductive performance. Synchronization of follicular waves and timed breeding are carried out effectively using GnRH protocol (Manjunatha et al., 2012). For best pregnancy results recipients should be synchronized so that they ovulate 24 - 48 hours after the donor. This can be achieved by random selection of the recipients from a group of camels and injecting them with hCG or GnRH 24 - 48 hours after the donor or by treating them daily with progesterone for 10 - 15 days and injecting eCG (1500 - 2500 i.u.) on the last day of progesterone treatment. This usually ensures the presence of

mature follicles in the recipient 24 - 48 hours after the donor. They can then be injected with hCG or GnRH to induce ovulation. Some of the major problems encountered with superovulation are poor response (overstimulation or ovulation failure) and refractoriness to the drugs. In addition; embryos yield and quality tended to be poor following superstimulation (Anouassi and Tibary, 2013). Embryos can be flushed and non-surgically transferred on day 7 after ovulation and pregnancy diagnosed by ultrasonographic examination of the uterus on day 17 - 20 of gestation.

To control ovulation; nowadays; natural mating, seminal plasma, LH, hCG, GnRH and vasectomized males are used to induce ovulation in alpacas, llamas and camels within 26-30 hours after treatment (Ratto et al., 2005; Skidmore and Billah, 2006, 2012; Rodrigues et al., 2012). Ovulation rate averaged 75% in vasectomized, 86% in GnRH and 100% in mating. Two injections of GnRH days apart, induced ovulation over 80%. Pregnancy rate of 80% has been achieved after ET into day 8. Pregnancy rates fall to 40% after transfer of frozen/thawed embryos using conventional slow-freezing or verification techniques (Skidmore et al., 2005)

In Vitro Embryo Production

In vitro production of embryos is an important reproductive biotechnology for the multiplication of genetically superior animals and the preservation of genetics. Techniques used include in vitro fertilization (IVF), intra-cytoplasmic sperm injection (ICSI), and embryo reconstruction using somatic cell nuclear transfer (SCNT or "cloning"). Methods for in vitro maturation (IVM); IVF and culture (IVC) are available for cattle; proved by the birth of innumerable calves worldwide (Galli et al., 2003). However; methods are still sub-optimal with respect to oocyte maturation (Merton et al., 2003; Lonergan, 2007; Russo et al., 2014) and the low oocyte yield per ovary (Machado et al., 2006). Velogenesis (e.g. IVM/IVF/IVC of prepubertal oocytes) has also been carried out successfully aiming the shortening of the generational interval., More efforts have to be made to optimize both repeated OPU retrieval and particularly, the current IVM procedures, which appears to be the major limiting factor for a satisfactory IVP at present. The sub-optimality and the costs related make these techniques of little application in cattle breeding;

particularly in developing countries. In camelids, relatively limited information is available on development of this technology. The production of embryos by IVM/IVF in camelids was first reported in llamas (Del Campo et al., 1994), however, the first offspring's were more recently produced in dromedary camel (Khatir and Anouassi, 2006).

In vitro maturation of oocytes for IVF; the first steps are collection and evaluation of cumulus-oocyte-complexes (COCs) and in vitro maturation of the oocyte. The majority of oocytes collected from slaughtered camel ovaries are in germinal vesicle, optimal culture time for maturation of dromedary camel oocytes was considered to be around 30- 32 h (Kafi et al., 2005; Wani and Nowshari, 2005). An incubation time of llamas COCs for 28-30h resulted in high maturation rates (Ratto et al., 2005). Tissue culture madiun-199 (TCM-199) is mainly being used for IVM of camelid oocytes. Maturation rates of 70 to 90% have been achieved (Tibary et al., 2011).

In vitro production of camel embryos has been reported using fresh ejaculated (Khatir and Anouassi, 2006) and stored epididymal sperm (Wani, 2008) with a development rate to the hatched blastocyst stage varies from 30 to 60%, pregnancy rates after embryo transfer are 30 to 40%, live birth rates from transfer of embryos produced by IVF is 20% and pregnancy loss is relatively high (Tibary et al., 2011). All the above studies have shown that the chronology of embryo development in camelids is faster than in other species irrespective of the source of spermatozoa used in IVF.

Embryo Splitting; Bisection and Reproductive Cloning

Cloning; as a multiplication technique, has been used in small ruminants since the late 1970s. Splitting of cattle embryos can be used to increase the number of embryos available from selected females and to produce genetically identical animals for biomedical research. Both separation of blastomeres in 2–4 cell embryos and embryo (morula or blastocyst) bisection have proven efficient to yield monozygotic twins after quick laparoscopic transfer to recipient cows. The overall efficiency of cow embryo splitting (number of calves born per embryos bisected and transferred) can reach almost 60%.

Nuclear transfer (NT) has been attempted and succeeded in small and large ruminants using blastomeres from 8 to 16 cells embryos, 32 cell embryos (goats) or sheep ICM-cells. Sheep was the first mammal to be

cloned from an adult somatic cell (Dolly, a sheep born in 1997 as the first cloned mammal). Calves have been successfully cloned via somatic cell nuclear transfer (SCNT), both using adult as well as foetal cells as nuclear donors; to produce transgenic animals for production of specific substances in milk. The effectiveness reached is still very low (Lee et al., 2004).

In camels, only 1 birth from cloning has been reported (Tibary et al., 2011). Production of embryos by NT has been reported in llamas; in which adult fibroblast cells were used as the nuclear donors (Sansinena et al., 2003).

Reproductive Emerging Technologies

One of them is sexing spermatozoa for directed production of offspring of a desirable sex by use of modified flow cytometric cell sorting of fluorescent dye-loaded living spermatozoa. The technology is, however, very promising and provides opportunities for sex selection of IVP-embryos, surpassing the need for sex diagnosis of the embryos (Blondin et al., 2009; Carvalho et al., 2010). Sex-sorting, is too costly (a flow sorter costs above 300 000 U$S), slow, and yields weak spermatozoa with reduced lifespan (Lonergan, 2007; Gosálvez et al., 2011).

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تقنيات التكاثر في الإبل

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تعمل تقنيات التكاثر علي تطوير كفاءة التناسل والوراثة وعلاج إنخفاض الخصوبة في مختلف الحيوانات.

تحتل الإبل مكانة فريدة في التناسل من حيث أن التبويض لا يتم إلا بعد الجماع. كما تنخفض الكفاءة التناسلية في الإبل بسبب اللزوجة المرتفعة لمني الجمال وقلة كثافة الحيامن بما يمثل عائقا كبيرا في تطوير وحفظ المني والإستخدمات الاخري لتقنيات التناسل.

يناقش المقال الحالي إنجازات التقنيات الحيوية في التناسل والتي تعرف بتقنيات التناسل المساعدة والتي تشمل علي: التحكم الهرموني للشبق والتبويض, حفظ الحيامن والأجنة , التلقيح الإصطناعي, الإخصاب الخارجي, نقل وإستنساخ الأجنة.

ويستعرض المقال الحالي الإنجازات في كافة مجالات تقنية التناسل السابق ذكرها والتأكيد علي اي من هذه التقنيات يصلح لتطرير التناسل في الإبل.