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Unlocking Camel Potential: Genetic Conservation through Modern Reproductive Technologies

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ABSTRACT

Camel breeding in Egypt is increasingly recognized for its potential, particularly due to camels' remarkable ability to adapt to harsh environmental conditions. Their unique physiological and morphological characteristics facilitate survival in desert habitats. As a result, numerous studies have focused on enhancing camel reproductive capabilities through various techniques, including ovulation synchronization, artificial insemination, and embryo transfer, which will be elaborated on in this review. A significant challenge in camel reproduction is the viscosity of semen, primarily influenced by seminal plasma, which results in uneven sperm concentration during dilution. Researchers are investigating different enzymes to effectively reduce this viscosity in the dilution media, achieving notable results. Nevertheless, further studies are necessary to improve reproductive performance in camels.

Keywords: camel, reproductive techniques, future prospects

INTRODUCTION

While successful genetic improvements in livestock are well-documented, research on the genetic variability of production traits in camels is relatively scarce (Dioli, 2016; Hemati et al., 2017). Genetic enhancements can also focus on commercially significant traits, such as racing performance and aesthetic qualities (Faye, 2015), or adaptations for machine milking (Ayadi et al., 2013). Current findings suggest that camels exhibit substantial genetic variability due to inadequate selection practices and their historical movement across regions (Almathen et al., 2016).

There is considerable potential within existing camel dairies globally to initiate genetic improvement efforts, as camels play a crucial role in the economies of millions residing in arid areas. Genetic enhancement in camels shows promise but necessitates collaboration among stakeholders and a better understanding of this remarkable species (Al Abri and Faye, 2019). Such knowledge is vital for grasping the distinctive biology of camels, which can aid in conservation and the development of genetic improvement strategies in Egypt and beyond (Sallam, 2020). Recently, Moawad et al. (2020) emphasized the importance of the dromedary camel (*Camelus dromedarius*) for its ability to produce high-quality meat, milk, and fibers in challenging environments. The reproductive performance of camels is closely tied to their capacity to produce these resources.

Over the past ten years, advancements in reproductive technologies, such as artificial insemination and embryo transfer, have surged (Basiouni, 2007; Quzy et al., 2013). The adoption of modern reproductive technologies in Old World camelids has increased, fueled partly by their unique reproductive traits and potential commercial applications (Faye, 2008), especially in regions where heat stress significantly impacts productivity, health, and fertility (Al Abri and Faye, 2019).

Reproductive Techniques in Camels

The significance of camel breeding in Egypt is becoming more apparent, especially in desert areas and newly developed regions. This is primarily attributed to their physiological traits that enable them to thrive in challenging environments. However, low reproductive efficiency remains a considerable limitation in the industry. Over the last two decades, various studies have aimed to enhance the reproductive performance of dromedary camels in Egypt through modern assisted reproductive technologies. Some of these technologies focus on male camels, particularly semen handling and processing, while others improve female reproductive efficiency through techniques like ovulation synchronization, artificial insemination, induction of ovulation, and early pregnancy diagnosis.

Environmental and physiological factors typically govern the onset and duration of the breeding season in species that exhibit seasonal reproduction. Research on dromedaries has, until recently, primarily focused on their racing potential, particularly in the Gulf region. However, significant efforts have been made to enhance reproductive efficiency due to various constraints affecting it (El-Hassanein et al., 2004). The breeding season for male camels in Egypt is relatively brief, usually spanning only 2-3 months from mid-December to late-February. This period is marked by distinct behavioral changes, such as increased neck gland activity, notable weight loss, the exteriorization of the soft palate, frequent urine spraying, and a characteristic shiny appearance in the hips (Zeidan et al., 2001; El-Hassanein et al., 2004; Bassiouni, 2007).

Testosterone, produced by Leydig cells in the testes, is crucial for stimulating later stages of spermatogenesis and maintaining secondary sexual characteristics and libido (Rateb, 2011a). In dromedaries, El-Bahrawy and El-Hassanein (2011) observed an average serum testosterone level of 2.9 ng/ml outside the breeding season, which rose to 7.9 ng/ml at the height of the breeding season, indicating increased sexual behavior.

Conversely, Rateb et al. (2011a) noted that testosterone levels in sub-fertile camels remained consistently low throughout the breeding season. The same researchers successfully altered the patterns of reproductive hormones, along with abnormal behaviors and semen characteristics, using gonadotropin-releasing hormone (Rateb et al., 2011b).

The effective application of assisted reproductive techniques (ARTs), such as artificial insemination, in vitro fertilization, and embryo transfer, relies significantly on the physical properties of collected ejaculates and their capacity for processing with minimal loss of fertilization potential. Evaluating semen quality is a crucial first step after collection, assessing its suitability for artificial insemination and the fertility potential of the camel bull. Due to the distinctive mating behavior of male camels, modified techniques typically used for other domestic species—have been developed (El-Hassanien, 2003).

The process of collecting semen from male camels involves the use of a female camel dummy, designed to closely mimic a real female during mating. An artificial vagina is positioned at the base of the dummy, enabling the operator to comfortably collect the semen from beneath it. This method reduces common issues associated with natural mating, such as injuries to the female and restlessness in the male, while also ensuring the safety of the operator.

Once semen is collected, proper handling and processing are critical for the success of assisted reproductive technologies (ARTs). Numerous studies have aimed at enhancing the quality of liquid-chilled and cryopreserved camel sperm. For instance, El-Bahrawy et al. (2006) assessed various diluents to find the most effective for preserving sperm quality during chilled and frozen storage, determining that a glycerolized tris-lactose medium (2% glycerol, v/v) was optimal. Additionally, El-Bahrawy et al. (2010) found that incorporating 400 μg/mL of ciprofloxacin significantly reduced microbial contamination in cryopreserved semen without negatively affecting post-thaw sperm quality. El-Bahrawy et al. (2012) later proposed a detailed protocol for semen collection and processing, suitable for laboratories handling camel semen, which included biweekly collection sessions, dilution of ejaculates with tris-lactose medium, and rapid thawing of cryopreserved doses (at 65°C for 10 seconds). More recently, El-Bahrawy (2017) reported that adding caffeine (4 mM) to the dilution medium enhanced post-thaw recovery of cryopreserved camel sperm and reduced the incidence of sperm abnormalities while preserving acrosome integrity.

Viscosity of Camel Semen

Dromedary camel semen is notably more viscous than that of other domestic species, primarily due to mucopolysaccharides and proteins in the seminal plasma, which are secreted by the bulbourethral gland or prostate during ejaculation (Mosaferi et al., 2005; Kershaw-Young et al., 2013;

Skidmore et al., 2013; Mal et al., 2016). This viscosity plays a crucial role in reproduction by preventing the backflow of ejaculate from the female's cervix, thus trapping sperm in a thick, condensed thread that decomposes slowly, allowing for a gradual release of viable sperm for successful fertilization (Deen et al., 2005; Vaughan and Tibary, 2006). However, high viscosity poses challenges for developing assisted reproductive techniques, as it leads to inconsistencies in sperm concentration during dilution, complicating effective mixing with cryoprotective components and straws for freezing (Deen et al., 2003; El-Bahrawy, 2010; Shekher et al., 2012). Thus, finding effective methods to reduce semen viscosity before processing is essential.

Over the last two decades, various strategies have been explored to mitigate viscosity, including standalone extenders (Wani et al., 2008) and mechanical methods like vortexing (Vaughan et al., 2003), stirring (Mosaferi et al., 2005; Niasari-Naslaji et al., 2007), needling, pipetting (Morton et al., 2008), and centrifugation (El-Bahrawy, 2010). Additionally, the use of enzymes in the dilution media has been widely investigated. For instance, Medan et al. (2008) utilized catalase, a hydrogen peroxide decomposition catalyst, while El-Bahrawy (2010) employed α-amylase to effectively reduce viscosity. Other studies have focused on the protein content in seminal plasma, demonstrating that protease enzymes like papain and bromelain can reduce viscosity while enhancing sperm motility and kinematics (El-Bahrawy et al., 2017). Both mechanical and enzymatic methods have been successfully combined for this purpose (El-Bahrawy, 2017). However, these proteases have been found to adversely affect sperm vitality and integrity, especially post-freezing and thawing (Rateb et al., 2019). An alternative approach using high-power, low-frequency ultrasound waves has shown promise in effectively reducing viscosity while preserving sperm characteristics with minimal adverse effects on vitality and membrane integrity (Rateb, 2016).

Enhancing Reproductive Efficiency in Female Dromedaries

The successful application of ARTs is heavily dependent on the reproductive physiological status of female camels. Ultrasonography has become crucial for monitoring ovarian activity and determining the phases of the ovarian cycle, as overt signs of estrus are often not apparent (Skidmore, 2011). Padalino et al. (2016) recently observed that during the ovulatory phase, female dromedaries exhibited specific behavioral patterns in the presence of a restrained male, which could serve as indicators of estrus. These behaviors included seeking the male's attention and displaying receptivity by staying close to him and assuming positions typical of natural mating. Generally, female dromedaries are seasonally polyestrous, with ovarian activity ceasing outside the breeding season, which in Egypt occurs from early December to late March. During this period, ovaries may remain inactive or contain only small recruiting follicles that rarely mature (Dholpuria et al., 2012; Padalino et al., 2016). Consequently, preparing female dromedaries for artificial insemination and embryo transfer outside of the breeding season has been a focus of recent research (Quzy et al., 2013).

Hormonal Induction of Synchronized Multiple Ovulation

Inducing multiple ovulations is vital for embryo transfer programs aimed at generating numerous follicles in donor females. Several hormonal protocols have been investigated for this purpose, both during (Vyas et al., 2004; Nowshari and Ali, 2005; Azizi-Moghadam, 2010) and outside the breeding season (Al-Sobayil, 2008). These protocols typically involve administering exogenous follicle-stimulating hormone (FSH) or similar hormones to stimulate ovarian activity (Anouassi and Tibary, 2013). For instance, administering a single dose of gonadotropin one day before or on the day of completing a progesterone treatment period has proven effective (Deen and El-Hassanein, 2013). Recently, Rateb et al. (2015) explored the potential of neutralizing endogenous inhibin to enhance ovarian dynamics during the transition period (mid-August to mid-December) in Egypt. Their findings indicated that active immunization against inhibin altered reproductive hormone patterns, stimulated ovarian follicular activity, and helped females overcome seasonal anestrus before the breeding season. Long-term observations showed that this immunization led to prolonged ovarian hyperactivity lasting five months post-treatment (Rateb et al., 2016). Furthermore, Khalifa et al.

(2016) developed a fixed-time induction regimen for synchronized multiple ovulation and insemination during the reproductive transition period in Egypt (mid-October to mid-November).

Monaco et al. (2017) also highlighted the benefits of using Lecirelin, a synthetic GnRH analogue, to facilitate ovulation in synchronized breeding programs for dromedaries. More recently, Khalifa et al. (2020) assessed the effectiveness of two hormonal protocols for inducing synchronized multiple ovulation in female dromedaries at the onset of the non-breeding season (April - May) in Egypt.

Artificial Insemination (AI)

Artificial insemination has been notably successful in Bactrian camels (Zhao, 1994; Zhao et al., 2000), but results for dromedaries have been less encouraging. The effectiveness of AI in dromedaries remains a subject of debate. Deen et al. (2005) reported pregnancy rates of 50-60% for artificially inseminated camels using fresh diluted semen within 30 minutes of collection, but this rate significantly decreased to 25-30% if the semen was used after 24 hours. Skidmore (2003) found no pregnancies resulted from using frozen-thawed semen, while using diluted-chilled or frozen-thawed semen yielded very low pregnancy rates compared to a 40% success rate when whole semen was applied (Deen et al., 2003).

Hormonal Management of Female Camels and In-Vivo Embryo Production for Embryo Transfer (ET)

Superovulation is a hormonal approach aimed at facilitating the release of multiple oocytes during ET programs, with the goal of generating numerous follicles in donor females (Kingsland, 2009). Anouassi and Tibary (2013) and Vettical et al. (2016) identified essential gonadotropic hormones for ovarian stimulation in camels, including Follicle Stimulating Hormone (FSH) and Pregnant Mare's Serum Gonadotropin (PMSG) or Equine Chorionic Gonadotropins (eCG), with dosages ranging from 1500 to 6000 IU for eCG and 400 mg for FSH.

Embryo transfer is a valuable method for promoting genetic advancement and enabling the international exchange of important breeding material while eliminating the need to transport live animals. It also plays a role in the conservation of endangered species (Amstislavsky, 2006; Niasari-Naslaji et al., 2009). Since 1990, the field of camel embryo transfer has seen significant progress. Skidmore and Billah (2005) highlighted the necessity of effectively inducing superovulation in donor females, as camels usually carry a single fetus, necessitating a well-coordinated synchronization program for recipient females. The camel racing sector has significantly fueled interest in ET, allowing competing females to bypass prolonged pregnancies and potential birthing complications (Tibary et al., 2005; Skidmore, 2013). Prior to participation in the program, both donor and recipient camels should undergo thorough health screenings and breeding soundness evaluations, including assessments for reproductive issues and major contagious diseases (Skidmore and Adams, 2000; Tinson et al., 2001; Anouassi and Tibary, 2013).

Non-Surgical Embryo Recovery

Embryo flushing is generally conducted using a non-surgical method similar to that employed in cattle. This process involves inserting a catheter into the uterus through the cervical canal, which can be achieved either by rectally manipulating the genitalia (Tibary and Anouassi, 1997) or by guiding the catheter into the cervix via the vagina (Anouassi and Tibary, 2013). An 18–22 gauge flushing catheter is used, with the uterus filled with flushing media that is then collected by gravity. Two primary methods are utilized: individual horn flushing or flushing the entire uterus with a balloon positioned just in front of the cervix. Approximately 800–1000 mL of flushing media is employed for each animal (Vettical, 2016). Additionally, the oviducts of donors can be flushed non-surgically while the animal stands, typically on days 7 or 8 post-mating (Abd-Elfattah et al., 2020).

In Vitro Embryo Production in Camels

In vitro embryo production (IVP) is a vital reproductive biotechnology aimed at increasing the population of genetically superior animals and conserving genetic material. IVP generally encompasses the in vivo or in vitro maturation of oocytes, followed by in vitro fertilization (IVF) and subsequent culture of embryos until transfer into the uterus (Farin et al., 2006; Fortunato and Tosti, 2011). The main goal of an oocyte maturation and IVF program (IVM/IVF) is to produce high-quality embryos that can successfully lead to normal pregnancies and live births upon transfer to recipients (Khatir and Anouassi, 2006). Although IVP techniques could enhance the number of embryos produced, their application in camels remains limited (Skidmore and Billah, 2005). Initial studies aimed to establish effective protocols for producing camel embryos in vitro, reporting success rates for reaching the blastocyst stage between 14% and 23% (Khatir and Anouassi, 2006; Abdoon et al., 2007; Wani, 2009; Khattab et al., 2020).

Research on using frozen semen from dromedary camels for IVF has indicated its effectiveness for embryo production (El-Sayed et al., 2012). The blastocyst rates were similar between frozen and epididymal spermatozoa during IVF (El-Sayed et al., 2012, 2015). However, further investigation is needed to address fertility issues related to preserved semen, despite its apparent high quality in vitro (Al-Bulushi et al., 2019). Currently, the efficiency of IVP remains low, with only 30-40% of oocytes developing into blastocysts, likely due to the in vitro environment's inability to replicate in vivo conditions, resulting in embryos with altered morphology and gene expression. Additionally, IVP may contribute to gestational disorders and impact offspring health (Abd El-Aziz et al., 2016). Despite three decades of research across various species, results in IVP remain inconsistent (Moawad et al., 2020).

Cloning in Camels

Cloning is generally regarded as inefficient, producing a limited number of viable embryos, which constrains its potential for large-scale agricultural applications (Al-Jassim, 2007). Nonetheless, its value lies in the ability to replicate genetically desirable animals, which could be crucial for the conservation of endangered species. Khatir and Anouassi (2008) initiated preliminary assessments of somatic cell nuclear transfer in dromedary camels. Notably, Wani et al. (2010) successfully demonstrated somatic cell nuclear transfer, resulting in the birth of a healthy cloned dromedary camel named Injaz.

Intra-Cytoplasmic Sperm Injection (ICSI)

Advancements in Intra-Cytoplasmic Sperm Injection (ICSI) techniques for certain domesticated animals offer promise for conservation efforts. The significance of ICSI in camel reproduction and the preservation of rare species is substantial. El-Sayed et al. (2015) confirmed the successful application of ICSI for producing embryos in dromedary camels. In 2018, Wani and Hong further improved embryo production via ICSI by chemically activating oocytes with ionomycin and roscovitine, enhancing cleavage rates and development to the blastocyst stage.

Future Prospects

Despite considerable progress and achievements over the past decade in improving dromedary reproductive performance, additional research is necessary in several areas. Optimizing the preparation and maturation of oocytes, as well as the capacitation medium for spermatozoa and culture media, is crucial for maximizing both the quantity and quality of in vitro-produced embryos. Furthermore, developing effective methods for selecting and isolating fertile spermatozoa from the ejaculate is likely to enhance outcomes in artificial insemination (AI), transvaginal embryo transfer (TE), and IVP. Additionally, establishing reliable cryopreservation procedures for camel embryos could broaden the practical applications of embryo transfer in camels.

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الملخص العربي

إطلاق العنان لقدرة الإبل: الحفاظ على الجينات من خلال التقنيات التناسلية الحديثة

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تحظى تربية الإبل باهتمام متزايد في مصر، وذلك لخصائصها الخاصة في مواجهة الظروف البيئية القاسية. فهي تتمتع بخصائص فسيولوجية وسمات مورفولوجية تسمح لها بالتكيف بشكل أكبر مع ظروف الصحراء. لذلك، أجريت العديد من الدراسات لتحسين القدرة التناسلية للإبل، مثل مزامنة التبويض والتلقيح الاصطناعي ونقل الأجنة، وغيرها من التقنيات التي سنناقشها في هذه المقالة ولكن أحد القيود التي تواجه تقنيات تكاثر الإبل هي لزوجة السائل المنوي. والتي تكون بسبب وجود بلازما السائل المنوي، والتي بدورها تؤدي إلى عدم التجانس في تركيز الحيوانات المنوية أثناء مرحلة التخفيف. عمل الباحثون على التخلص من لزوجة السائل المنوي من خلال استخدام العديد من الإنزيمات المضافة إلى وسائط التخفيف واكتسبت الأبحاث التطبيقية الكثير من النجاح. ومع ذلك، هناك حاجة إلى المزيد من الموضوعات للتركيز عليها، للحصول على مزيد من التحسن في الأداء التناسلي للإبل.

الكلمات الدالة: الإبل، تقنيات التكاثر، آفاق المستقبل