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Animal Biotechnology for Livestock Production 1

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Sustainable agriculture is a rapidly growing field aiming at producing food and energy in a sustainable way for humans and their children. Sustainable agriculture is a discipline that addresses current issues such as climate change, increasing food and fuel prices, poor-nation starvation, rich-nation obesity, water pollution, soil erosion, fertility loss, pest control, and biodiversity depletion.

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Preface

Many developed countries are actually facing a ban of animal food products, promoted mainly by urban and vegetarian activists who have never experienced living in farms and who do not acknowledge that their modern way of life is largely the result of hard work of their elders with the help of farm animals. However, since the start of society, animal production has been an essential agricultural sector worldwide providing food, labor, aesthetics and social values, and even today many farmers would not survive without animals. This book entitled 'Animal Biotechnology for Livestock Production 1' is our first volume providing advanced knowledge on biotechnological methods to improve the livestock production, with focus on animal reproduction, health, diagnosis and nutrition. Chapter 1 presents on artificial insemination in cattle, with focus on physiology aspects of the estrous cycle, estrus synchronization program, ovulation synchronization program for timed artificial insemination, strategies for improving fertility and use of sexed semen in artificial insemination. Chapter 2 reviews biotechnological applications for production of dromedary camels, with details on camel herd reproduction, reproduction control and artificial insemination. Sperm dilution, thawing, conservation, and insemination techniques are also discussed. Recent biotechnological applications for livestock production are summarized in Chap. 3, with emphasis on somatic cell nuclear transfer, artificial insemination, embryo transfer, embryonic stem cell technology and marker assisted selection.

Cattle production in France. Copyright 2021 Eric Lichtfouse

Chapter 4 reviews applications of stem cells in livestock, with emphasis on mesenchymal stem cells. Immunomodulatory, antimicrobial activity, migration and reparative functions of stem cells are detailed. Chapter 5 presents techniques for profiling proteins and metabolites associated with feed efficiency in dairy cattle. Recent findings on key metabolites and proteins of metabolic pathways are also disclosed. Chapter 6 focuses on processing, packaging, and safety of dairy products. Applications of biotechnologies in food diagnosis are also explained. Chapter 7 reviews 'on-farm point-of-care' diagnostic technologies in animals. This chapter covers various point-of-care and on-farm diagnostic technologies for monitoring animal health and disease with focus on molecular, electrochemical-biosensors diagnostics. Chapter 8 presents biotechnological applications in the poultry industry. This chapter covers the concepts and developments of biotechnologies for poultry production, breeding, feed and nutrition. This chapter also discusses applications in poultry vaccines, biologics, disease diagnosis and food processing.

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Chapter 2
Reproduction Management and Artificial Insemination in Dromedary Camel

Djallel Eddine Gherissi and Ramzi Lamraoui

Abstract The dromedary camel is a unique multipurpose species under extreme arid conditions. It is used for production, leisure, transport and agricultural work. Its profile as a production animal has given it major importance in ensuring the sustainability of marginalized communities in the poorest regions of the world, but also because of the nutritional and therapeutic properties of its milk and meat. Nowadays, the exploitation of this animal resource known an orientation towards intensification under the pretext of the expanding demand on camel products. The productivity of camel herds is conditioned by its reproductive potential. Often, camel herd performances are limited by low fertility rate, high inter-calving intervals, late puberty leading to low longevity, low milk production and unavailability of young calves for herd renew and fattening farms. To reach an optimal level of camel herd's numerical productivity and therefore accelerate the genetic progress, it is imperative to reduce the duration of the unproductive periods, namely the waiting period and the reproduction period. This involves through the choice of a suitable farming system, improving breeding practices, improving data recording and reproductive monitoring, application of strategic reproduction control and new reproduction techniques.

Here we review strategies of camel herd reproduction management and means to improve camel herd reproductive performances using sexual activity control in males and females and artificial insemination practice. Controlling ovarian cycles of the female camel consists of controlling follicular growth and timing of ovulation. Therefore, this technique can be used to induce and synchronize the ovarian function during a favorable reproduction period or even during the non reproductive

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season. Zootechnical means do not really make possible to synchronize the ovarian cycle in camels. They are used as complementary tools to the pharmacological methods. The different hormonal based protocols are: melatonin, progesterone, prostaglandin F2α and GnRH. Artificial insemination in dromedary camel is a topic of contemporary research that tends to develop standard sperm collection protocols, monitoring of sperm quality, short-term preservation, cryopreservation and semen thaw with considerable interest to increase the pregnancy rate and therefore to market this technology in camels for large-scale use. Various genetic, sanitary and economic advantages could be insured by this biotechnology and overcome many problems in regard with camel fertility efficiency such as short breeding season, long gestation period, traditional reproductive management and widespread of genital and venereal infectious diseases.

Keywords Artificial insemination · Oestrus synchronization · Dromedary camel · Camel bull · Breeding · Reproduction performance · Sperm · Follicular cycle · Female camel · Calving interval

Abbreviations

°C	*degrees Celsius*
AF	*annual herd fertility*
AI	*Artificial insemination*
AV	*artificial vagina*
BLUP	*Best Linear Unbiased Prediction*
CASA	*Computer Assisted Sperm Analysis*
CFM	*Calving to first mating interval*
CI	*Calving interval*
CIDR	*Controlled Internal Drug Releasing*
COI	*Calving-oestrus interval*
DC	*dromedary camel*
eCG	*equine chorionic gonadotropin*
EDTA	*Ethylenediaminetetraacetic acid*
FAO	*Food and Agriculture Organization*
GnRH	*gonadotropin releasing hormone*
GPG	*GnRH-PGF2 α-GnRH*
h	*hour*
hCG	*human chorionic gonadotropin*
IIED	*International Institute for Environment and Development*
ILRI	*International Livestock Research Institute*
IMV	*Institut de médecine vétérinaire*
INRA	*Institut national de la recherche agronomique*
LH	*luteinizing hormone*
M1, M2	*Maiting1, Maiting 2…*

mA	*ampere*
min	*minutes*
ml	*millilitre*
NGF	*nerve growth factor*
OD	*Open days*
OIF	*ovultion inductor factor*
PGF2α	*Prostaglandin F2α*
PHT	*Progeny History Testing*
PRID	*Progesterone Relasing Intravaginal Device*
RP	*reproduction period*
SeNPs	*Selenium nanoparticles*
SSO	*small and smooth ovaries*
V	*volt*
WP	*waiting period*
ZnONPs	*Zinc oxide nanoparticles*

2.1 Introduction

The dromedary camel (DC: *Camelus dromedarius*) is the best livestock animal well adapted to arid ecological conditions. It fights against desertification by using a high floristic biodiversity with high digestibility and better food efficiency. In addition, its low gregarious outpatient pasture and long intestinal transit contribute for a better germination of the seeds after excretion. Camels are multipurpose animals well adapted to harsh environment. They are used for nutritional, dietetic, economic, social and cultural purposes. They offer milk and meat production, hair and are used for racing, transportation and tourism (Al Abri and Faye 2019). The nutritional value of camel meat has been shown to be much higher than beef, lamb and goat because it contains less fat with a comparable index of essential amino acids (Raiymbek et al. 2015). Camel meat is rich in essential amino acids such as leucine and lysine, essential fatty acids such as omega 3, minerals, vitamins and bioactive compounds such as carnosine, anserin, glutathione, etc. (Kadim Isam et al. 2018). Camel milk is 3–10 times richer than cow's milk in vitamin C. (Faye 1997; Konuspayeva et al. 2009; Al Abri and Faye 2019). Its hypo-allergic property is due to its low content on β-casein and β-lactoglobulin (Konuspayeva et al. 2009). It's whey protein fractions are higher (El-Agamy 2006). So, dromedary camel contributes to the subsistence of transhumant and nomadic Saharan communities, often with restricted access to various protein resources of animal origin. Thus, it constitutes for them an element of food security in the face of climate change, a long-term capital ensuring the well-being of the family and an element of social prestige. Finally, the dromedary participates in sports and leisure activities and represents a cultural heritage in many countries and contributes in socio-economic activities and

diversification of many marginalized area of arid areas worldwide (Al Abri and Faye 2019).

Poor reproductive performance in camel species is mainly due to a low fertility rate and increased inter-calving interval. The low numerical productivity of camel herds is often discussed among cameleers with adversarial opinions in regard to its aspects and its risk factors. However, the veterinarian should provide advice based only on specific information with reference to herd reproductive history. The serious difficulty often arises is about the reproduction evaluation in DC herds is often confronted to the following constraints: a pastoral breeding system in continuous displacement, traditional breeding practices and lack of recording information related to reproduction, absence of state veterinary services interventions on reproduction associated to difficulties to reach remote breeding areas for regular monitoring by private veterinarians, lack of appropriate training for health and management of camel herds. The diagnosis and treatment in this animal are generally approached in the same way as for other farm animals.

The reproduction control in camel species implements technical procedures or herd management strategies that make possible optimizing reproductive performances, particularly during anoestrus period (seasonal, post-partum) taking into the count the breeding systems of the species. It aims to reduce the rate of infertility, choice of calving period, calving synchronization, and decrease of unproductive periods (advancement/synchronization of puberty, decrease of anoestrus length, mating and conception during the non rutting season). Efficient methods to reproduction control in the camel are required as the interest grows in the potential application of artificial insemination and embryo transfer as tools for improving genetic traits, such as milk, meat and wool production and racing ability (Al Eknah 2000).

Compared to other farm animals, little research on camel reproduction control and artificial insemination has been found in the camel side. Oestrous synchronisation solves oestrous detection problems and makes artificial insemination more effective (Helmy 1991; Minoia et al. 1992). In female camels, ovulation may happen through intrauterine semen deposition without the need for coitus (Chen et al. 1985; Musa et al. 1990). This makes AI a reliable technology for continuous genetic advancement using males of high genetic potential and inseminating female groups that have undergone synchronization of follicular development. However, the large-scale use of this biotechnology faces many difficulties, such as semen selection, male camel sexual activity, heterogeneity of recovered semen quality (small volume, low sperm concentration and high viscosity) (Skidmore et al. 2013), limited knowledge of semen storage, optimum insemination time and sperm dose (Al-Bulushi et al. 2018).

2.2 Reproduction and Genetic Improvement of Dromedary Camels

The DC (*Camelus dromedarius*) is known for its rather long reproductive cycle characterized by a long prepubertal period, long gestation period, long interval between generations and a low fertility rate (Gherissi et al. 2020a). Likewise, the incidence of genital pathologies, abortions and neonatal mortality are high (Gherissi et al. 2019, 2020a, b). All these factors together lead to a low numerical productivity of camel herds, particularly in extensive breeding system (Faye 2018; Brigitte 2005).

Genetic improvement according to the classical method involves the animal identification, assessment of their performance, selection of breeders and use of breeders in order to achieve genetic progress. In fact, genetic improvement can only take place if performance and pedigree are recorded. Selection would be effective when it is carried out following estimation of breeding value using progeny (BLUP: Best Linear Unbiased Prediction) widely practiced in various animal species.

$$BV_p = 2\left(\frac{n}{n+k}\right)\left[P\left(progeny\right) - CA\right]$$

$$k = \frac{4-h^2}{h^2} \qquad r^2 = \frac{n}{n+k}$$

BVp: breeding value of the parents, r^2: reliability, n: number of offspring (informants), P (progeny): Mean value of the performance of the progeny, CA: Comparison average which characterizes the environment.

According to the previous formula, the precision of the selection depends closely on the quality and quantity of the individual controls of the available informants and the herd size used to calculate comparison average of the performance. Thereby, the low reproduction levels within camel herds can generate serious difficulties in order to give to appreciate with great reliability the genetic potential of the parents who will serve as reproducers (Emami Mibody et al. 2016). Contrariwise, good herd reproduction statue make easier to appreciate genetic value and animals with the highest predicted value can be selected as parents with minimum error.

On the other hand, genetic progress level depends on the variance of the genetic value in the population, the intensity of selection, the reliability of the estimated breeding value and the generation interval. In this regard, Al-Sobayil et al. (2006) and Almutairi et al. (2010) showed that camels species have a high genetic variability reflected in the heritabilities of various traits (body weight and growth rates were moderate to high; $h^2 = 0.24–0.40$, birth weight $h^2 = 0.37$, Daily gain ranged $h^2 = 0.25$ and 0.49, milk yield at 305 days $h^2 = 0.24$ and test day yields $h^2 = 0.22$). This reflects a potential for ample genetic gain if systematic selection is to be implemented (Al Abri and Faye 2019; Bahbahani et al. 2019).

$$GP \quad per \quad year = \frac{S_A \cdot i \cdot r_{VE,\widehat{VE}}}{t}$$

GP: genetic progress, S_A: variance of the genetic value in the population, i: the intensity of selection, $r_{VE,\widehat{VE}}$: reliability of the estimated breeding value t: generation interval.

The selection intensity was indicated by the proportion of animals required for the next generation to be parents. Reproductive efficiency and management of camel herds therefore have an important influence on the rate of parents necessary for the next generation to be produced and, therefore, on the rate of genetic progress. The generation interval in this species is long (approximately 8 years) and is highly dependent on genetic potential, environmental conditions, husbandry systems and breeding methods. A higher reproduction rate for a given population size means a lower number of breeding animals and, therefore, a higher selection intensity. A greater number of offspring per breeder also promotes a more accurate estimate of genetic values (Emami Mibody et al. 2016). Another benefit of increased reproductive rates is the faster dissemination of superior genetic material. In this context, reproduction control, in particular using reproductive biotechnologies, can have a direct effect on increasing the rate of genetic progress of camel herds.

Artificial insemination (AI) leads to high reduction in the use of breeding males. In dromedaries, male and female camels with high genetic values could constitute a nucleus of selection of less than 1% of the entire population. AI increases the available information on siblings and therefore increases the genetic value accuracy estimated in their parents (Van Arendonk and Bijma 2003). Furthermore, AI allows increasing the selection intensity and precision of males based on the progeny test especially for traits with low heritability (such as functional traits). The semen exchange between different herds allows for the establishment of genetic links between them and reduces the risk of venereal diseases transmission between herds. Sperm freezing gives organizations and AI centers ability to create camel gene banks as back-up stores of camel genetic diversity. In addition, cryopreservation would facilitate the exchange and transport of semen and the international exchange of camel's genetic material.

AI requires availability of technical skills in AI centers and camel farms with effective means of communication between these two stakeholders. However, in many camel breeding countries, financial means are lacking and the structuring of camel breeding is inadequate for setting up successful AI operations. In addition, most camel herders are reluctant to camel breeding intensification and prefer mobile and pastoral systems which make difficult to follow the follicular cycle and implement timed AI at large scale. Therefore, AI is probably not predictable for livestock in extensive grazing systems.

2.3 Reproductive Cycle of Female Camels

The female camel is strict monotocus and seasonal polyoestral breeder (Akral and Khanna 1995). During the non breeding period the sexual behavior disappears but the follicular waves persist at low growth levels (Gherissi et al. 2018) similar to what happen in ewe (Dogan et al. 2020) but different to mare who undergoes a deep ovarian rest in the non breeding season (Boeta et al. 2006). Ovulation is induced by GnRH-like factor or Ovulation inductor factor (OIF) contained in camel bull seminal plasma which is identified as nerve growth factor (bNGF) (Sanjay et al. 2012; Bogle et al. 2012) also with luteotrophic effect on the corpus luteum (Silva et al. 2014). This molecule induces pre-ovulatory luteinizing hormone (LH) surge 2–3 h after mating (Marie and Anouassi 1986) followed by ovulation (32–40 h after copulation) and subsequent formation of corpus luteum (El-Allali et al. 2017). Without mating at the breeding period (mature follicle of 11–19 mm); female's exhibit repeated cycles of follicular growth and regression without a cyclic corpus luteum. Three main phases have been described for follicular wave in female camel (Fig. 2.1): growth, maturity and regression. After emergence of small follicles

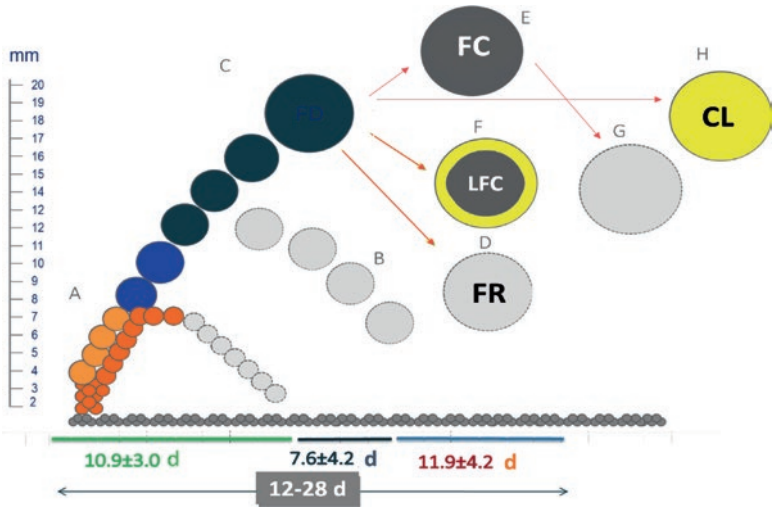

Fig. 2.1 The main follicular cycle phases in female camel (*FC* follicular cyst, *LFC* luteinized follicular cyst, *FR* dominant follicle regression, *CL* corpus luteum)

Following this first recruitment phase, several follicles (3–6) continue to grow (5–17 mm) until the emergence of one or two large dominant follicle which becomes mature; including 5–10 mm medium size follicles and 11–17 mm pre-ovulatory follicles. The dominant follicle undergoes a stagnation stage for 5–7 days. In 50% of unmated females, regression occurs until disappearance in the ovarian stroma. In other unmated females (50%) the largest follicle continues to grow attaining a large size (30–65 mm); this cyst-like follicle cannot ovulate spontaneously however, it regresses thereby permitting the expression of the next follicular growth waves in an average of 18 days later. In mated females, following ovulation, the mature follicle is usually transformed into a functional corpus luteum. (Adapted from Zarrouk et al. 2003)

(<4 mm in diameter) at the periphery of the ovaries during the recruitment phase, several follicles (3–6) continue to progress as medium size follicles (5–10 mm) until the dominance of one or two large follicles (11–19 mm) that become(s) mature pre-ovulatory follicle(s). The growth phase (<4 mm–10 mm) extend over 10.9 ± 3.0 days and dominance phase lasts over 7.6 ± 4.2 days (Skidmore et al. 1994).

Follicular regression occurs in 50% of unmated females until total desperation of these follicles in the ovarian stroma. The largest follicle tends to develop in other uncoupled females (50%) to reach an oversized follicle (>25 mm), which constitutes a follicle cyst that decreases ovulatory response (Tibary 2018). Presence of 2 or more co-dominant follicles in camelids is not rare and can occur in up to 40% of the follicular wave (Manjunatha et al. 2015; Campbell et al. 2015). A combination of stimuli, including the chemical factor in the seminal plasma, neurohormonal responses to the mechanical stimuli of coitus and the male effect, could result in the ovulatory response in the camel (Al Eknah 2000).

The anovulatory hemorrhagic follicles cysts seems to be the most frequent among ovarian cysts in female camels (Gherissi et al. 2019; Tibary 2018). The pathophysiology of anovulatory hemorrhagic follicles is poorly understood and their presence seems to not disturb follicular wave patterns (Tibary 2018). The follicular cyst or the corpus luteum of the non pregnant females regresses 18 days and 11 days later, respectively (Fig. 2.1), allowing the next waves of follicular growth to begin (Zarrouk et al. 2003; Marie and Anouassi 1987). The total length and the seasonal frequency of follicular wave in the female camel was found to vary considerably (17–28 days and maintain or reduced frequency of follicular waves) according to the geographic location, nutrition statute, start and end of the breeding season, body condition, photoperiod (Abdoon 2001; Vyas et al. 2004; El-Allali et al. 2005; Sghiri and Driancourt 1999; Gherissi et al. 2018, 2020b). In mated females, the ovulated follicle is usually transformed into a functional corpus luteum and its maintenance is conditioned by the suppression of PGF2α release before day 10 after ovulation (Skidmore et al. 1998).

2.4 Camel Herd Reproductive Performance Evaluation

2.4.1 Data Collection for Reproduction Analysis

Obtaining individual reproductive history is integral to an accurate evaluation of reproductive herd performances and to explain eventual difficulties that arise there. Regarding the reproductive particularities under pastoral camel breeding conditions, a method based on data collection from breeder's offspring history called "Progeny History Testing" (PHT), has been suitably adapted to camel reproduction investigations. This technique was developed firstly in Ethiopia by a team from the Ministry of Agriculture, and then widely used by the International Livestock Research Institute (ILRI). PHT method was approved by the International Institute

for Environment and Development and the World Food and Agriculture Organization (IIED and FAO) in their data collection guides for different farming systems.

Under semi intensive management system, identification of all the animals in the herd using the most relevant means to camel species is essential. The breeding management in general and reproduction in particular must be done in compliance with certain preliminary conditions relating to the animal and its environment leading to monitoring and data collection by the breeder himself and stakeholders, in particular by taking observations, clinical examinations and preventive or curative treatments. The observations recording in semi-intensive camel farms is a concept that is absent in almost all camel breeders. This preliminary practice for the evaluation of the reproductive status of farms is fundamental and must be encouraged by using clear or even cryptic language. A mutually agreed system of abbreviation ratings can be very useful. Such information is manifold; they are particularly linked to calving (day, number, nature, type, interventions, etc.), sexual cycle (postpartum cycle number, number of cycles used, etc.) mating (days, duration of sexual receptivity, mating time, mating after induced or natural oestrus), pregnancy diagnosis (day, nature, result), body condition score, pathologies (absence of oestrus, ovarian and uterine pathologies, infections, embryonic mortality, mastitis, etc.). All observations must always refer to the identity of the animal. It will be accompanied by the date or even the time of the observation as well as the nature of the observation. The use of recording, scoring and organizing observations for processing is highly recommended. The most modest would be a simple farm management camel breeding calendar. Computerized systems adapted to camel breeding could emerge, which would effectively improve the management of several other aspects related to reproduction such as milk production, nutrition, etc.

2.4.2 Reproduction Indicators of Camel Herds

2.4.2.1 Calving Interval (CI)

The CI is the most important criteria to look at when talking about reproduction of the camel. The lengthening of this parameter leads to increase unproductive period (even drying period). It also decreases the herd numerical productivity by decreasing offspring production per year. Finally, it increases reform rate and herd replacement cost. Therefore, camel herders have every interest in the fact that their female camels give births often with the shortest intervals throughout their whole life.

In terms of herd performance; the interest is to measure the average of this parameter over the entire present females in order to identify the multiple individual and collective reasons for its lengthening/shortening. It is possible to shorten the CI of females by acting on waiting period (WP: time between calving and first mating) and reproductive period (RP: time between the first mating and the conception) (Fig. 2.2). WP can be shortened by rigorous peri-partum management with particular attention to late pregnancy and parturition (preparation, obstetric assistance and

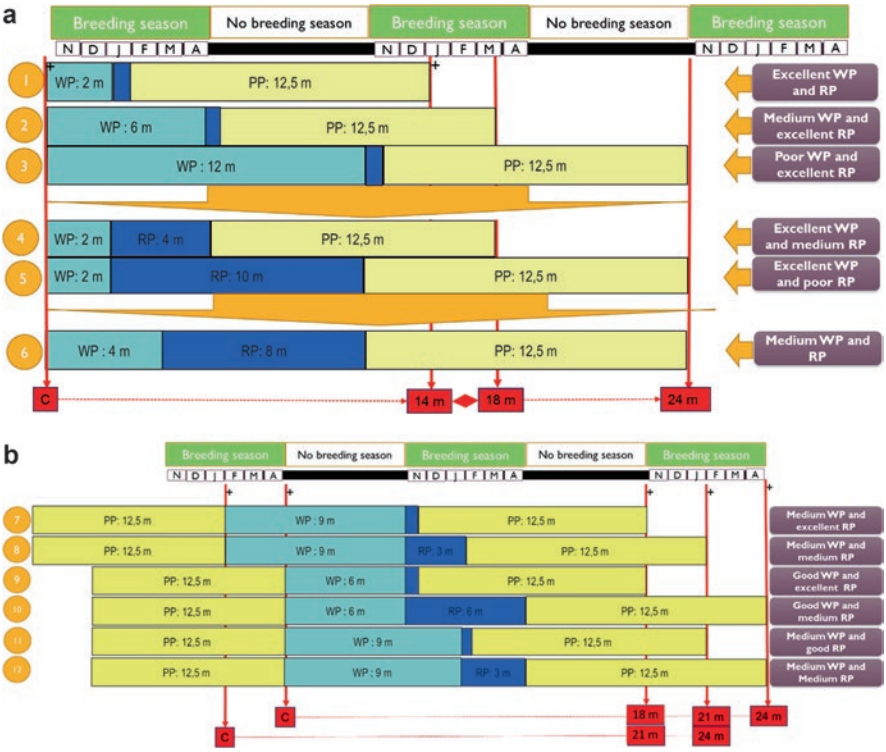

Fig. 2.2 Potential factors responsible for calving interval (CI): 18 months as objective and 24 months as threshold when calving occur at the beginning (**a**) or the middle/end (**b**) of the breeding season. WP: Waiting Period, RP: Reproduction Period, PP: Pregnancy Period. Calving at the beginning of the breeding season results on 2 to 12 months period of open days (OD) and females can achieve CI equal or less than 24 months. Excellent WP and RP lead to OD of about 2 months and CI of 14 months (box 1). Medium WP or RP lead to OD of about 6 months and CI of 18 months (boxes 2 and 4). Females with poor WP or RP will have an OD of 12 months and CI of 24 months (boxes 3 and 5). This last situation (CI of 24 months) could occur when moderate reproductive difficulties affect both WP and RP (Medium WP and RP, box 6). Animals with difficult reproductive status show association of anoestrus and infertility that lead to poor WP and RP and lead to OD and CI exceeding 12 months and 24 months (out of threshold), respectively. However, when the ovarian resumption and/or uterine involution periods overlaps with the seasonal anoestrus, the CI would be approximately 18–24 months. This is especially true when calving occurs on the middle or at the end of the breeding season. In this case, medium WP with excellent to good RP would give CI of 21–24 months (boxes 7, 8, 11 and 12). Similarly, the calving interval would be 21–24 months if there is a good WP with excellent to medium RP (boxes 9 and 10). In case of prolonged anoestrus associated with subfertility, the CI would exceed 24 months (out of threshold). (Adapted from Gherissi et al. 2020a)

balanced nutrition) and using good management practices (inputs and offspring's weaning). Calving at the beginning of the breeding season coincides with the wet period and food availability, which promotes a good body condition and early resumption of ovarian activity with mating and pregnancy during of the same

season (Fig. 2.2). Regular checkups of postpartum animals to diagnose and treat related health problems (including mastitis, uterine infections, intercurrent diseases) and stillbirths are also recommended during WP. The RP management must be implemented by a regular balanced supply diet during the late pregnancy and postpartum period, camel bull flushing and sexual behavior and fertility control (male/female ratio, reform due to 'infertility …), as well as the specific diagnosis and treatment of genital diseases (metritis, bursitis …) (Tibary et al. 2005; Ali et al. 2010).

2.4.2.2 Calving-Oestrus Interval (COI)

Healthy female camels show first postpartum oestrus between 14 and 42 days under pastoral livestock systems (Elias et al. 1984). When female camels were mated between 5 and 6 weeks post partum, adequate conception rates were obtained (Derar et al. 2014). The COI could, however, be extended to the next breeding season when calving occurs in the middle or at the end of the breeding season. Taking into account these factors, the COI target was set at 2 to 6 months and the threshold at 9 months (Gherissi et al. 2020a; Table 2.1). Open days (OD).

High rates (70–80%) of cyclic females were reported at 30 days postpartum in well-fed dromedaries, therefore the conception rates at 45 days postpartum were about the average scores (Tibary and Anouassi 1997a; Derar et al. 2014), but ovarian cyclicity can resume after 8–10 months when the diet is inappropriate for ovarian functioning (Skidmore et al. 1994). Therefore; when female camels conceive within the same calving season OD would be less than 6 months. However, if the dam is mated during the next breeding season OD period could last, instead form 7 to 12 months. Accordingly, the objective for this parameter was set at 180 days and the threshold at >360 days (Gherissi et al. 2020a, Table 2.1).

Table 2.1 Standard objectives and thresholds of reproductive parameters of female camels

Variable	Unit	Objective	Threshold
Age at first oestrus	Months	24–36	>42
Age at first mating	Months	24–36	>48
Birth to conception interval	Months	36–48	>54
Age first calving	Months	48–54	>60
Calving to oestrus interval	Months	2–6	>9
Open days	Days	≤180	>360
Calving interval	Months	≤18	>24
Annual fertility	%	>85	<75
Mating-pregnancy diagnosis	Days	21	30
Pregnancy length	Months	12.5	13.5
Culling age	Years	15–17	> 17
Calving number	Integer	5	$7 > n > 3$
Culled females per year	%	<5	>30

Adapted from Gherissi et al. (2020a)

2.4.2.3 The Annual Herd Fertility AF (Pregnancy Rate)

It reflects the ratio within a year between the number of pregnant female camels and the number of mated females. The fertility rate for camel herds looking to improve camel numerical productivity should rages between 70% and 95% (Faye 2018). The agreed objective and threshold for this parameter are >85% and <75%, respectively (Gherissi et al. 2020a).

2.4.2.4 Female Camel's Calving Interval Objective and Threshold

The CI depends waiting period (postpartum to first mating), reproduction period (first mating to conception) and the female's fertility. Calving to first mating interval (CFM) in pastoral conditions occurs within 14 to 42 days (Elias et al. 1984). Derar et al. (2014) showed that camels could be mated between the fifth and sixth week postpartum with satisfactory open days and conception rates. The time to first oestrus (associated to first mating) after calving varies from 1 to 38 months with mean value of 10.3 ± 5.77 months (Gherissi et al. 2020a). High levels of CFM would be due to poor nutritional conditions leading to late resumption of follicular activity that takes up 8–10 months (Gherissi et al. 2018). The pregnancy duration in female camel is about 12.5–13 months (Gherissi et al. 2017).

The prompt ovarian resumption and occurrence of first mating earlier in the same calving season allow considering CI objective of 14–18 months and alarm threshold of 24 months (Fig. 2.2a). If calving occurs in the middle or late breeding season, the CI target would be 18–21 months and the alarm threshold would be 24 months (Fig. 2.2b). In fact, the objective CI is set at ≤18 months and the alarm threshold is set at >24 months (Table 2.1).

2.5 Reproduction Control in Dromedary Camel

Reproductive control tools can reduce unproductive periods by extending breeding season length and stimulating follicular growth and inducing ovulation during the non breeding season. They facilitate allow rational herd management to improve animal's resilience to harsh livestock condition under arid environment (calves care, fodder availability and postpartum control) and to adapt herd production according to market needs (milk, fattening and calves). In addition, they allow accelerating genetic progress, firstly by facilitating the widespread dissemination of AI and secondary by developing ovarian stimulation protocols and embryonic transplantation.

Synchronisation of ovarian activity principles applied in cows are used to synchronize ovarian activity in female camels, but methods are adjusted to physiological and behavioral characteristics of camelids, resulting in varying degrees of performance. The reproduction control in female dromedary camels is possible

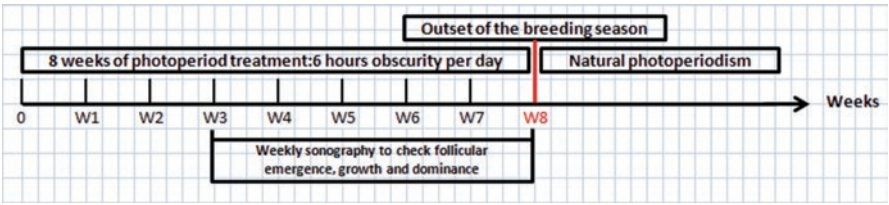

Fig. 2.3 Photoperiodic treatment of female camels 2 months before the beginning of the breeding season

using zootechnical means such as photoperiodic control (Vyas et al. 2008; Swelum et al. 2018a), food supplementation (Hammadi et al. 2001), male effect (Hafez and Hafez 2001) or hormonal exogenous supply such as melatonin (Dholpuria et al. 2012; Swelum et al. 2018b; Ainani et al. 2018; El-Allali et al. 2018) or progesterone (Monaco et al. 2013; Swelum and Alowaimer 2015; Swelum et al. 2018c), GnRH and PGF (Quzy et al. 2013; Al-Sobayil 2003, 2008; Manjunatha et al. 2018). The different applied protocols would be followed by blind natural mating or AI in fixed time using fresh or frozen semen.

2.5.1 Photoperiodic Control

In female camels, the photoperiodic control consists in applying a blindfolded eyes using black mask 6 h per day beginning 2 months before the start of the breeding season (November) (Vyas et al. 2008). Darkness perception by female camels using photoperiodic control leads to melatonin synthesis and release which advances the date of the sexual season. This remarkably induced a good follicular growth activity from the third week of treatment and the development of the ovulatory follicles between fifth and seventh week of treatment (Fig. 2.3; Vyas et al. 2008). Likewise, the ovulation, mating and pregnancy rates are quite considerable (Vyas et al. 2008).

In camel bulls, significant increase in testicular measurements and volume, libido, melatonin and testosterone concentrations were achieved by blindfold application during the non-breeding season to induce a shortened daily photoperiod of approximately 2.55 h relative to the natural day cycle (Swelum et al. 2018a).

2.5.2 Male Effect

Just one study was found in literature, documenting the pheromonal "male effect" causing female camels to cycle earlier in the breeding season (Hafez and Hafez 2001). In fact, the use of the male effect in camel species is rarely considered. Behavioral and endocrine processes of this practice to control reproduction in

camels are by far to be compared with other species such as sheep and goats. These two species are spontaneous ovulation while the camel is characterized by induced ovulation through mating.

2.5.3 Flush Feeding

The daily feed supplementation using 4 kg of commercial concentrated food during the late pregnancy (about 2 months before calving) and at the start of the postpartum period (5 kg/day for 3 months) helps to improve the female camels overweight at the time of calving and allows a rapid and significantly higher rate of ovarian activity resumption (Hammadi et al. 2001), short waiting period (PA) (around 40 days), high rate (approximately 70%) of females mated for the first time at 60 days after parturition (Hammadi et al. 2001) and high conception rate (Mostafa et al. 2016a, b). In addition, this practice helps to better growth of camels up to the age of 90 days (Hammadi et al. 2001).

2.5.4 Ultrasound-Guided Aspiration of the Follicle

The elimination of the dominant follicle using ultrasound-guided fine needle aspiration makes possible to end the current follicular wave and emergence of new follicular pool in an average time of 2.3 ± 0.5 days. The dominant follicle deviation occurs after 8.8 ± 1.1 days (Skidmore et al. 2009).

2.5.5 Exogenous Melatonin Based Protocols

The effectiveness of treatment based on exogenous melatonin consists of mimicking short days either at the beginning or at the end of the breeding season. For female dromedary camels; two melatonin treatment methods are possible. The first option is to administer it 2 months ahead the natural breeding season to support the follicular waves emerging and ultimate growth of the dominant follicle(s) at the start of the breeding season (Dholpuria et al. 2012). For example, in Algerian local condition it carries out at early September and continues until the end of October over 8-week period (Fig. 2.4). The second option is to start treatment at the end of the breeding season to allow an ovarian follicular activity extension for the rest of the year (non breeding season) (El-Allali et al. 2018). This treatment begins at early April and ends in late May (Fig. 2.5). At the end of these two proposed treatments, monitoring of ovarian activity is recommended to ensure the quality of ovarian cycles.

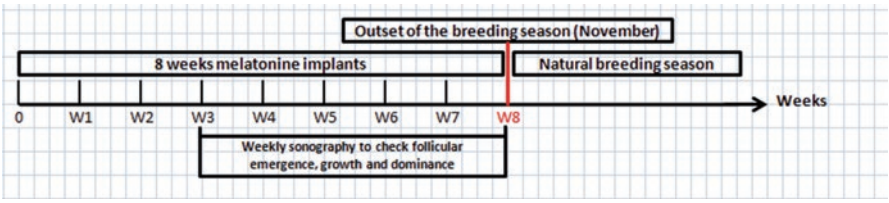

Fig. 2.4 Exogenous melatonin treatment of female camels 2 months before the beginning of the breeding season

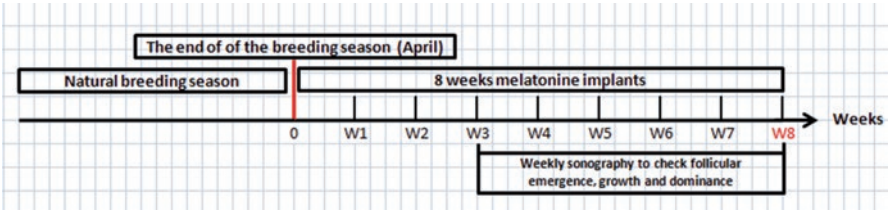

Fig. 2.5 Exogenous melatonin treatment of female camels 2 months after the end of the breeding season

To administer exogenous melatonin for camels, Dholpuria et al. (2012) recommend to use commercial implants containing 18 mg of melatonin at a dosage of 1 implant per 28 kg bodyweight (approximately 20 implants per female camel), which to be inserted subcutaneously by a 12-gauge applicator, preferably sterilized beforehand (Fig. 2.6).

For camel bull; the treatment during the non breeding season with melatonin implants (18-mg per 28 kg of live bodyweight) or approximately 28–32 implants per bull two times at an interval of 35 days helps to improve significantly their reproductive performance (Plasma testosterone, testicular measurements, sexual behavior and spermogram) (Swelum et al. 2018b).

2.5.6 Exogenous Progesterone Based Protocols

Daily progesterone injection (100–150 mg) and various devices secreting progesterone (CIDR 1.38 g: Controlled Internal Drug Releasing Device, new PRID Δ 1.55 g: Progesterone Relasing Intravaginal Device) are experimented to synchronize follicular waves in female camels (Cooper et al. 1990, 1992; Monaco et al. 2013; Swelum and Alowaimer 2015; Hussein et al. 2015; Swelum et al. 2018c; Tibary 2018; Abo El-Maaty et al. 2019). Cleaning of perineum and flushing of vagina with iodopovidone solution before device insertion is very important since it clinically improves vaginal environment at the end of the treatment (Monaco et al. 2013). Female camels receive CIDR for 7 days (Abo El-Maaty et al. 2019), 10 days

Fig. 2.6 The technique of application of subcutaneous melatonin implants in camel. (From Dholpuria et al. 2012)

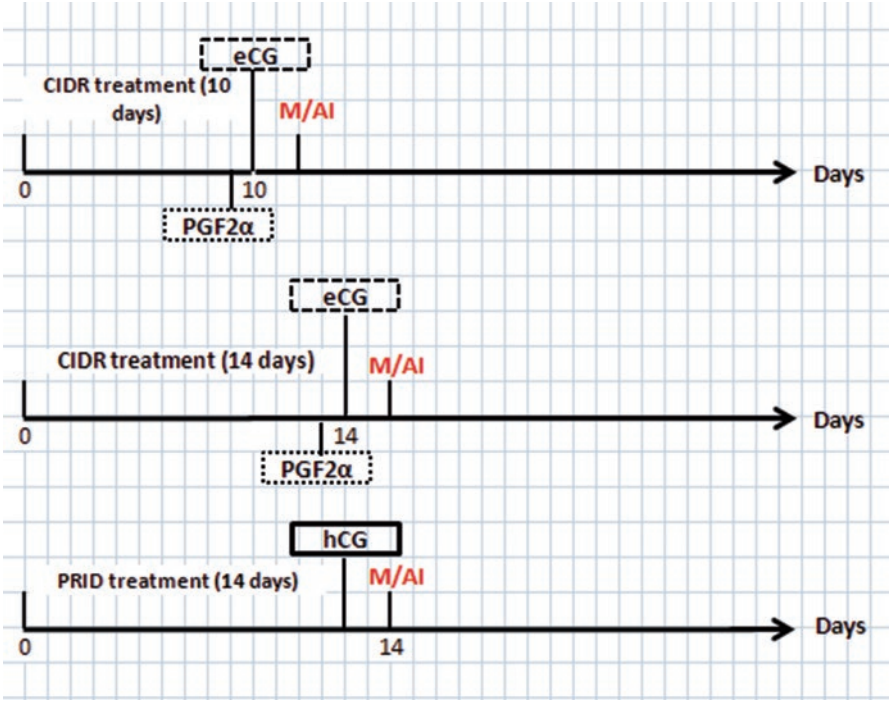

Fig. 2.7 Exogenous progesterone treatments during intra-vaginal devises (CIDR, PRID) with eCG or hCG and timed artificial insemination

(Monaco et al. 2013, Fig. 2.7), 14 days (Swelum and Alowaimer 2015; Al-Fatlawi and Al-Hamedawi 2017; Fig. 2.7) or 17 days (Al-Sobayil 2008), while new PRIDΔ is held 14 days for Swelum et al. (2018c; Fig. 2.7). The progesterone plasmatic concentration increases earlier after device insertion (2 days) but its plasmatic peak is observed after 12 days (Swelum and Alowaimer 2015).

There are conflicting studies on the effectiveness of PRIDs and CIDRs (Monaco et al. 2013; Tibary 2018). The authors conclude that the treatment of exogenous progesterone could fully prevent sexual receptivity and suppress the development of large follicles without suppressing follicular wave's growth. These devices have sometimes been related with increasing spontaneous ovulation (Tibary 2018).

There are conflicting reports on the efficacy of PRIDs and CIDRs (Monaco et al. 2013; Tibary 2018). The authors opinions converge that exogenous progesterone treatment could completely prevent sexual receptivity and suppresses the growth of large follicles but could not suppress the follicular wave. Sometimes, these devices have been associated with increased spontaneous ovulation (Tibary 2018). Injection of PGF2α (500μg) one day before CIDR withdraw (Monaco et al. 2013), eCG (2000 IU) at the day of CIDR withdraw (Al-Sobayil 2008) or hCG (3000 IU) at the last day of PRID treatment (Al-Sobayil 2008) were also practiced in female camels (Fig. 2.7). The daily injection of progesterone (100 mg, IM) for 10–16 days provided promising results in multiple ovulated female camels (McKinnon et al. 1994; Tibary 2018). More comprehensive studies are necessary to better understand the effectiveness of different associated treatments to exogenous progesterone administration on follicular wave synchronization in female camel. A recent study found that long-acting injections of progesterone can be used in camels and can be more effective than daily injections (Chhaibi et al. 2016). Synchronizing follicular waves using progesterone devices reminds a controversial issue between the authors particularly on their effects at different breeding seasons (Tibary 2018). Their effectiveness at the beginning and in the middle of the breeding season has been reported vain due to its action in reducing the follicular diameter but not follicular number (Monaco et al. 2013). During the breeding season Swelum and Alowaimer (2015) reported a high percentage of camels presenting ovulatory follicles 2–4 days after CIDR withdrawal showing therefore its significant interest in follicular wave synchronization. In another more study; Swelum et al. (2018c) confirmed the interest of new PRIDΔ in the synchronization of follicular waves during the breeding season with a high rate of females which carry ovulatory follicles 2 days after the end of treatment. The ovulation rate, the non-return rate are significantly increased, the open days period reduced mainly and the fertility rate is higher when the blind natural mating or timed AI are practiced 48 h after PRID withdraw (Al-Sobayil 2008; Al-Fatlawi and Al-Hamedawi 2017; Swelum et al. 2018c).

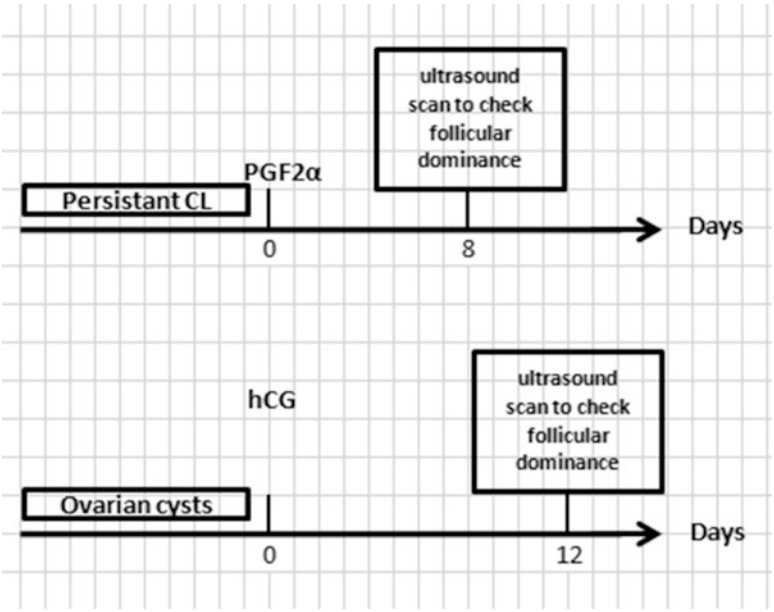

Fig. 2.8 Prostaglandine (PGF2α) and human chorionic gonadotrphin (hCG) treatments of persistent corpus luteum and ovarian cysts, respectively

2.5.7 Prostaglandin and Human Chorionic Based Protocols

PGF2alpha (500μg) administration for female camels with a persistent CL and hCG (4500 IU) for those with cystic ovaries were reported to have high efficacy on the number of females with ovaries showing ovulatory follicles 8 and 12 days post-treatment, respectively, but the pregnancy rate for both protocols remained low (Quzy et al. 2013; Fig. 2.8).

2.5.8 GnRH-Based Protocols

"*Select synch*" treatment 2 months ahead the natural breeding season with GnRH (250μg) on day 0 and PGF2α (5 ml) on day 7 (Skidmore et al. 2009) or day 10 (Mostafa et al. 2016a, b) did not improve the quality of follicular wave at the end of the treatment (Fig. 2.9). GnRH alone may be effective in synchronizing follicular waves to mitigate the harmful effects of prostaglandin on the antioxidant potential of camels and their response to breeding (Abo El-Maaty et al. 2019). Follicular wave emergence and follicular deviation occur about 3 days (range 2.5 and 3.5 days) and 5.5 days (range 4 and 11 days) respectively, after GnRH administration

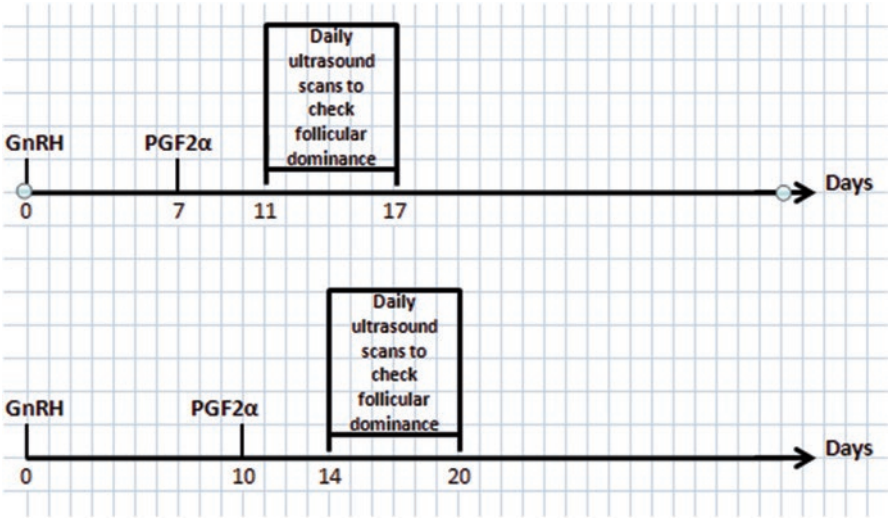

Fig. 2.9 Select synch (GnRH-PG) protocol for oestrus induction in female dromedary camels

(Manjunatha et al. 2015; Skidmore et al. 2009) and 4.5 days and 10.7 days for GnRH-PGF2α treatment.

The Ovsynch (GnRH-PGF2α-GnRH: 0–7-9) and eCG (2000 IU) treatments for female camels with small and smooth ovaries (SSO) 2 months ahead the natural breeding season induced high effective rates of animals with matures follicles at day 8 and day 12 post treatment, respectively (Quzy et al. 2013; Fig. 2.10). The GPG (0–7-14) treatment showed high proportion (≈73%) of female camels had a total of 22 dominant follicles (1.3–1.9 cm) of which 21 ovulated after GnRH injection day 14 (Skidmore et al. 2009; Fig. 2.10).

Ovsynch (0-7-10- 22:M1) protocol with timed natural mating followed by pregnancy diagnosis 21 days later and ovarian ultrasonographic monitoring of non pregnant females for second timed mating was examined by Manjunatha et al. (2018) with high rate of follicular wave synchronization and consistent pregnancy rate (Fig. 2.11).

Ovsynch -Resynch (GPG-M1-GP-M2; Fig. 2.12) treatments were experimented recently by Manjunatha et al. (2018) in dromedary camels during the breeding season. Firstly these authors used Ovsynch protocol (GnRH-PGF2α-GnRH: 0-7-10) followed by timed mating 12 days later (day 22). Then, systematic GnRH injection was received by all female camels at day 36 regardless to their pregnancy statue; followed by pregnancy diagnosis at day 43. PGF2α (day 43) was administrated to non pregnant females which were resubmitted to second timed mating at day 48. This protocol showed high proportion of ovulated female camels after Ovsynch protocol. The GnRH in Resynch protocol resynchronized effectively the follicular development for second mating of non pregnant females with high early and late pregnancy rates and low rate of pregnancy loss (Manjunatha et al. 2018).

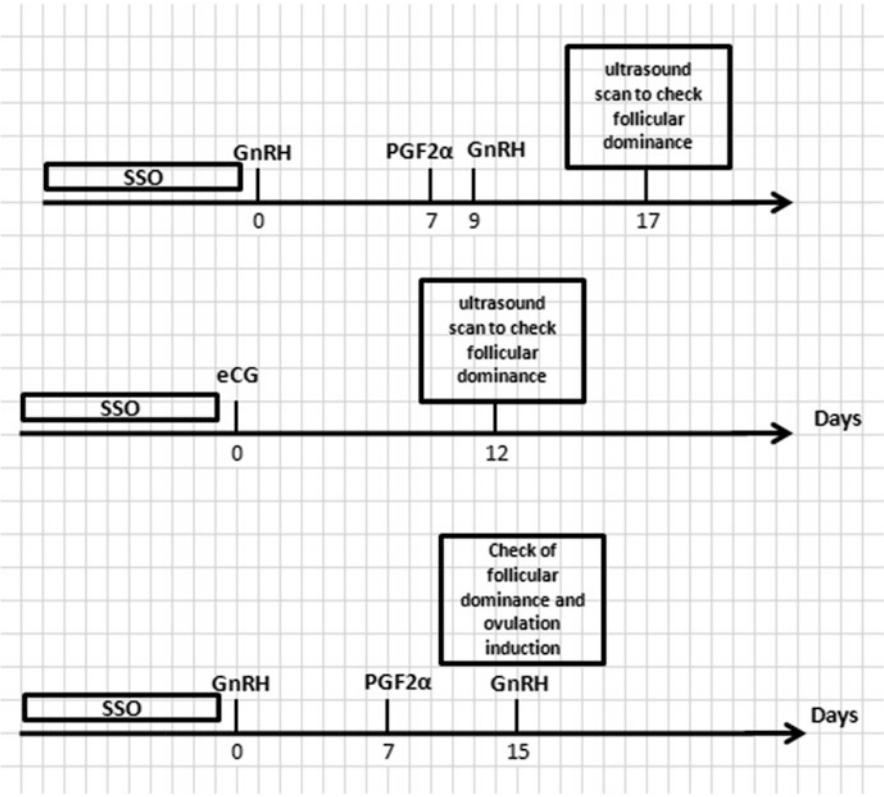

Fig. 2.10 Ovsynch (GnRH-PG-GnRH) and eCG (equine Chorionic Gonadotropins) protocols for oestrus induction in female dromedary camels with small and smooth ovaries (SSO) 2 months ahead the natural breeding season

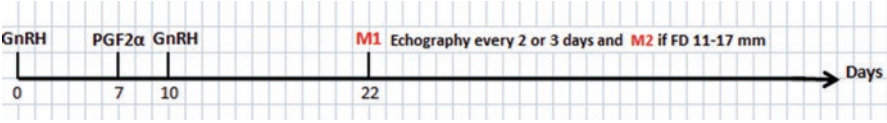

Fig. 2.11 Ovsynch (GnRH-PG-GnRH) protocol followed by blind natural mating and ultrasonographic monitoring of non pregnant females for second timed mating

The repetitive follicular wave resynchronization (GPG-M1-GP-M2-GP-M3; Fig. 2.12) after each mating was also explored by Manjunatha et al. (2018). They reported high synchronization rate at first mating and also high resynchronization rate at second and third timed natural mating. The pregnancy rate and the pregnancy loss rate after Ovsynch-first mating and Resynch protocols-second and third mating showed very interesting results (Manjunatha et al. 2018). An encouraging pregnancy cumulative rate is favorable to adopt this protocol in camels under farm and field conditions (Manjunatha et al. 2018).

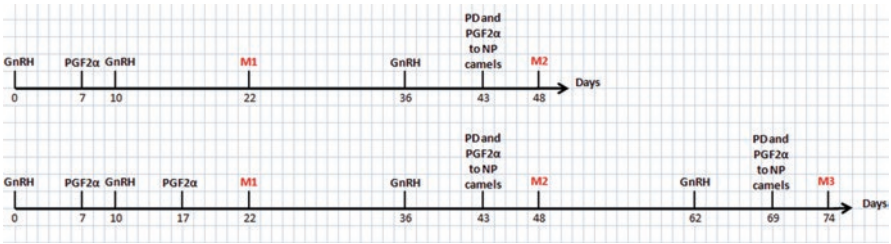

Fig. 2.12 Ovsynch -Resynch (GnH-PGFGnRH-M1-GnRH-PGF-M2) protocol for oestrus synchronization of female dromedary camel

The listed reproductive control methods could help to improve the CI in camel herds and therefore decrease the unproductive periods, overcome the constraint of the short breeding season, facilitate herd management practices (vaccination of mothers, maintenance and growth of camels, weaning, postpartum control …) and thus improve the birth rate and the numerical productivity, milk and meat production and in the long term decrease the generation interval for accelerating genetic progress.

2.6 Artificial Insemination

2.6.1 Definition

Artificial insemination (AI) is the act of depositing sperm by means of an instrument, at the most opportune time and in the most suitable place in the female genital tract. Since the 1960s, the use of AI as a breeding technique in camelidae has been reported with first camelid offspring from Bactrian camel obtained by this technique in 1961 (Elliot 1961). However, this technique has gained popularity over the last 25 years as interest is increasing to improve camel genetic characteristics such as milk, meat and wool quality, as well as racing ability. AI in DC faces several problems, including complications related to semen collection, bull sexual activity, semen quality (low volume, low sperm concentration and high viscosity), limited awareness of optimal insemination time and sperm dosage per insemination session, and lack of a standard storage technique (Elliot 1961; Tibary and Anouassi 1997c; Al-Bulushi et al. 2019b).

2.6.2 Importance of Artificial Insemination in Camels

The method offers many advantages: on the one hand, it facilitates the management of reproduction in the camel herds and increases the reproductive capacity of the males (ratio male/females) and therefore to obtain the most offsprings which helps rapid genetic improvement. On the other hand, it is a preventive measure against sexually transmitted diseases. Moreover, the use of AI would prevent the need to transport male or female animals for mating, thus reducing the charges and danger of moving valuable animals. The short and long term sperm conservation allows the international transportation of semen and conservation of the genetic material of males with high genetic level. It helps eliminate behavioral problems so AI would minimize the risks of injury. Finally, it facilities the application of cross breeding programs between different camelid's species.

Table 2.2 Advantages and disadvantages of sperm collection using artificial vagina

Advantages	Disadvantages
High sperm concentration	Decreased sperm mobility with prolonged contact of sperm with the inner membrane of the AV
High frequency of sperm collection during periods of sexual activity	Requires a trained camel bull
Not very aggressive technique, preserves the well-being and the natural sexual instinct	Requires high technicality of the operator
Allows observation of sexual behavior to assess male reproductive ability	Injuries risk of the animal and the operator
Can be replaced by candom device or held between the female's hind legs	Risk of refusal mating and especially when using dummy or with AV poorly suited
Can be used in the presence of female teaser in estrus or a camel dummy	High cost associated with the use of teaser females
Collection can be facilitated by creating an underground room or small laboratory underneath the dummy	
Method adapted to the insemination center: high collection rate, high sperm quality, less stressful manipulation of animals with high genetic potential, collection hygiene	
More adequate for short term and long term sperm preservation	
Higher pregnancy rate with fresh raw sperm	

Table 2.3 Advantages and disadvantages of sperm collection using an electroejaculator

Advantages	Disadvantages
Sperm collection during non breeding season	Low sperm concentration
More safe for the animal and operator	Requires pharmacological tranquilization of the camel bull
Lower cost	Aggressive technique that does not take into account the sexual instinct and comfort of the male camel
Collection of males with certain conditions that do not allow normal mating (locomotor diseases)	Low frequency and cadence of sperm collection
	More difficult sperm conservation
	Lower pregnancy rate

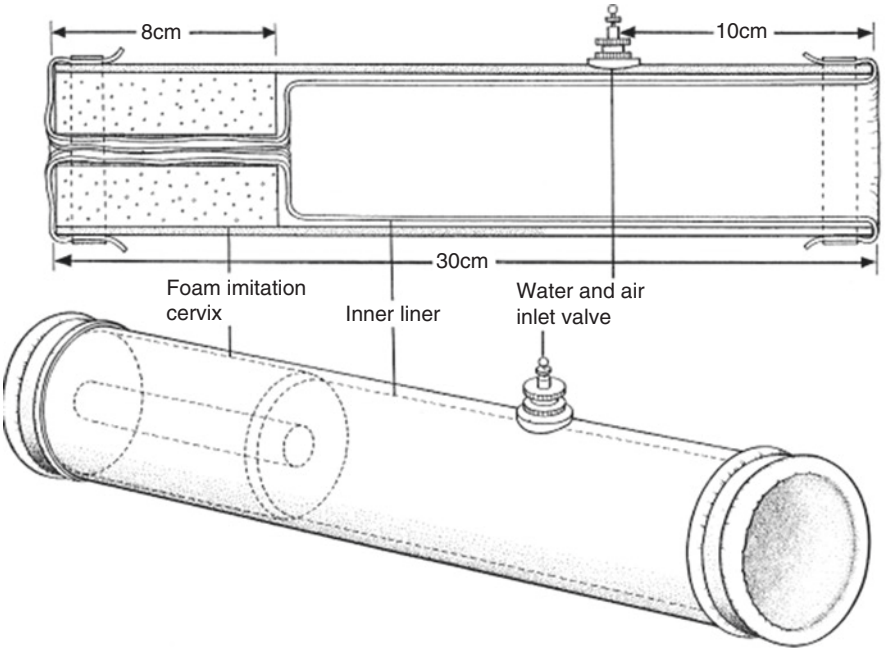

Fig. 2.13 Diagram of an artificial vagina used for DC. (Reprinted with permission of editor: Aurich Christine; from Bravo et al. 2000)

2.6.3 Semen Collection

The semen collection is the first step in AI and/or its examination. The primary objective of a semen collection is to obtain a large number of clean and optimum quality ejaculates (Monaco and Lacalandra 2019). The collection method has mighty effects on the physical and possibly biochemical properties of the ejaculated material (Tables 2.2 and 2.3) (Tibary et al. 2014). The artificial vagina (AV) and

Fig. 2.14 Semen collection of male DCbull using modified bull-artificial vagina and female camel teaser with operator who direct penis by hand manipulation of sheath towards the opening of the artificial vagina. (**a**) Modified artificial vagina for collection of semen, (**b**) Coil on short artificial vagina to simulate cervical rings and (**c**) Collection of semen on a receptive female mount. (Photos reprinted with permission of the editor: Ahmed Bamouh, from Tibary and Anouassi 2018)

electroejaculation are the accepted methods for semen collection in camels (Bravo et al. 2000). The AV constitutes the means widely accepted by several authors who have worked on the dromedary using a female camel as a teaser or dummy. It's simple and practical device which has two parts. An outer cylinder made of rigid material, most often hard and thick rubber (thermal insulation) or plastic with an opening closed by a stopper. The inner latex or artificial rubber is introduced into the outer cylinder and its ends folded down and held in place by an elastic band. The cavity thus is formed by the outer cylinder and the inner liner. One end of the artificial vagina is lubricated: it will be used to introduce the penis; on the other is fixed a rubber cone at the end of which is fitted a graduated glass or better plastic tube to collect the sperm (Figs. 2.13 and 2.14).

Electroejaculation is rarely applied in the camel species (Tibary et al. 2014; Tibary and Anouassi 2018). This procedure requires the tranquillization or even the

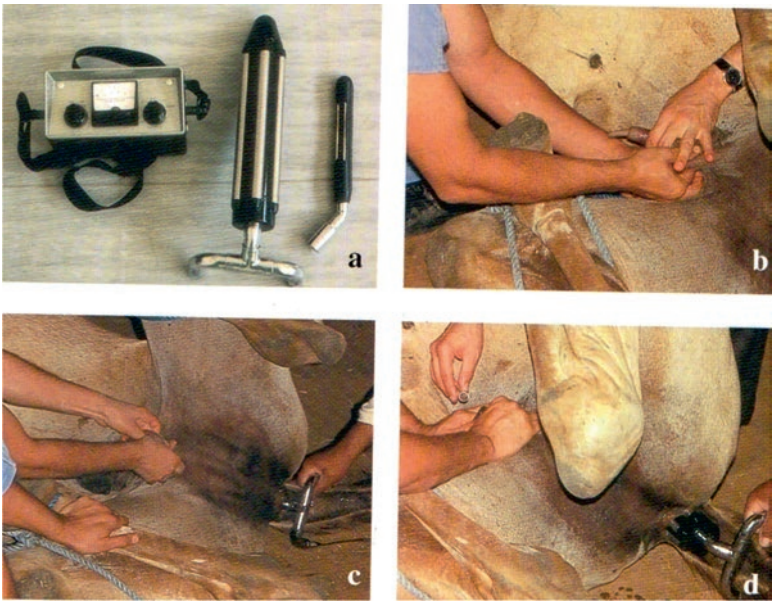

Fig. 2.15 Semen collection of male dromedary camel bull using an electroejaculator. (**a**) Electroejaculatro, (**b**) exteriorization of part of the penis outside the sheath, (**c**) placement of the electroejaculator after animal preparation, (**d**) semen collection step. (Photos reprinted with permission of the authors: Ahmed Tibary and Abdelhak Anouassi, from Tibary and Anouassi 1997b)

sedation of the animal, which would present a health risks (Fig. 2.15). The degradation of the physico-chemical quality and the concentration of the sperm collected by electroejaculation make it difficult its conservation and freezing (Tingari et al. 1986). So, electroejaculation (12V, 180 mA) can be applied with exceptional cases with camel bulls presenting highly vigorous and aggressively behavior, with low libido or with certain physical problems and for collection of semen during the non breeding season (Skidmore et al. 2013; El-Hussanein 2003). In this chapter, we don't consider this technique because its use does not meet the requirements of animal welfare.

Old methods of collecting semen such as collecting sperm directly from the vagina and collecting sperm by massaging the genital pool glands have also been reported in dromedaries (Tibary and Anouassi 1997b).

The modified bull-AV was commonly used for camel bull with good results (Bravo et al. 2000; Tibary and Anouassi 2018; Fig. 2.13). Some precautions must be taken into consideration in order to adapt the AV and avoid causing injuries to male bulls leading to the refusal of the animal for subsequent samples. The short modified AV allows also keeping away sperm from contact with the rubber liner of the AV and avoiding soiling the sample that adversely affect sperm quality. Therefore, it is recommended to:

- Shortening AV to allow the semen to pass directly into the collection flask. The internal part would have a diameter of 5 cm and a length of 30 cm (Bravo et al. 2000). The internal sleeve ends with a rubber receiving cone on which the collecting tube will be fixed. The whole will be secured with two rubber straps.
- To prevent contact with the rubber material, an additional removable plastic inner liner can be added, but the authors have found that males do not tolerate this well (Skidmore et al. 2013).
- For a perfect stimulation of ejaculation in dromedaries, the modified bull-AV should be equipped by foam imitation cervix of about 8 cm in length at its distal part. This provide a narrowing simulating cervical to receive the highly mobile urethral process of the camel penis to stimulate ejaculation during the extended copulatory (Bravo et al. 2000; Monaco et al. 2016; Monaco et al. 2018; Skidmore 2019). The filling water (55–60 °C) must be sufficient and it is necessary to blow air between the inner liner and the outer rigid wall to achieve an inner temperature of 38–40 °C and a pressure equal to that of the female vagina (Skidmore et al. 2013).
- The filling water (55–60 °C) must be in sufficient quantity and blowing air between the inner liner and outer rigid wall are important to obtain an internal temperature of 38–40 °C and a pressure equivalent to that of the female vagina (Skidmore et al. 2013).
- Keeping the vagina in an oven at 45 °C and fill it only in the minutes preceding the sample. Overpressure is not recommended because it may not leave enough space for the penis during ejaculation and cause the internal wall to burst. Correct filling is when thr upright opening of AV simulates a vaginal slit.

The semen collection should be done as much as possible in a quiet place to avoid any stressful situation for the animal and on non-pulverulent soil for the comfort of the operator and to avoid contamination of the sample as camels mate in a couched position. The use of a female teaser is essential. The impact of time of female teaser presentation and number of mating attempts before going to effective semen collection seem to have important effect on collected sperm volume and concentration (Al-Bulushi et al. 2018. These factors were reported to have a significant effect by increasing male excitation and improving sperm quality in other species (Hanzen 2015).

When the animal is in mating position, the penis will be directed by means of the operator hand applied to the sheath towards the opening of the artificial vagina, which directed slightly outwards while avoiding any excessive deviation of the penis. The sensory arousal of moist heat drives the intromission reflex. Before ejaculation is done, the male will make multiple thrusts, interspersed by periods of rest. The ejaculate typically happens in fractions and whole process can be prolonged between 5 and 50 min (Bravo et al. 2000). Thus, it is very difficult to know if ejaculation has occurred; collection continues until the male stands up. The effective ejaculation (intromission into the artificial vagina until the end of ejaculation) takes place after a variable duration ranging from 5 min to 20 min (Anouassi et al. 1992; Hassan et al. 1995) without yet a correlation between the duration of collection and

the quality of the sperm. Soon after, the male stands up. Since the process of semen collection might be quite long, addition of about 1–2 ml of extender into the collection vessel before collection may be beneficial (Bravo et al. 2000). Immediately after the male stands up, the vagina is turned over so as to collect the sperm in the collection tube. The latter will, if necessary, be protected from any thermal shock by wadding or another isothermal envelope.

On the other hand, sexual peculiarities of the camel species (Tibary and Anouassi 1997a, b, c; Bravo et al. 2000; Monaco et al. 2018); such as the copulatory behavior ie, mating in sternal recumbency, lengthy ejaculation throughout copulation (from 5 min to 50 min), aggressive sexual behavior, the highly viscous nature of the semen and intrauterine semen deposition constitute major constraints to sperm collection and have made it mandatory to adapt sperm collection techniques with various degrees of success. We particularly mention:

- Using some specific hosing and welfare conditions to prevent injuries, stress and stereotypical behaviours (oral, locomotor, self-ejaculation) associated with poor
- reproductive performance (Monaco and Lacalandra 2019).
- Enhancing endocrine pathways and ejaculation quality of the male sexual by acting on the herd structure in which is maintained the camel bull, photoperiodic treatments and/or pharmacological (exogenous melatonin) protocols (Monaco et al. 2018; Monaco and Lacalandra 2019).
- Using female camel teaser and placed in sternal recumbency position with the operator in an underground room beneath the collection area which allows full observation of mating behavior and ejaculatory pattern (Hemeida et al. 2001; Fig. 2.16).
- Using artificial vagina devices implanted under the female teaser (Tibary and Anouassi 1997a, b, c; Al-Eknah et al. 2001; Fig. 2.17) or mounted on a dummy (El-Hussanein 2017; Fig. 2.18).
- Replace teaser female by a dummy designed in the similar position for natural mating, inside which is inserted an AV. The dummy is fixed on the floor of the collection yard and as a ceiling for a small laboratory to exchange AV from one

Fig. 2.16 Semen collection of male dromedary camel bull using artificial vagina oriented and maintained by an operator in an underground room which allows him full observation of mating behavior and ejaculatory pattern. (Photos from Hemeida et al. 2001)

Fig. 2.17 Semen collection of male dromedary camel bull using artificial vagina held between the female's hind legs. (Photo reprinted with permission of the authors: Ahmed Tibary and Abdelhak Anouassi, from Tibary and Anouassi 1997b)

Fig. 2.18 Semen collection of male dromedary camel bull using artificial vagina mounted in a camel dummy. (Photo reprinted with permission of the editor: Daryoush Babazadeh, from El-Hussanein 2017)

male to the other and to carry out rapid evaluation and partial extension of the collected semen (El-Hussanein 2003, 2017; Ziapour et al. 2014; Fig. 2.19).

- Place condoms within the vagina of female teasers which showed acceptable result regarding ejaculated sperm volume and its characteristics (Tibary and Anouassi 2018; Fig. 2.20).
- Training camel bull to semen collection using dummy device and condoms. In this regard, serious behavioral limitations were reported if males are not used exclusively for AI (Tibary and Anouassi 2018; Monaco and Lacalandra 2019).
- To prevent semen contamination, a rope circled around the thorax could be used to attach the tail and the prepuce could be washed and dried before beginning the semen collection session (Monaco and Lacalandra 2019).

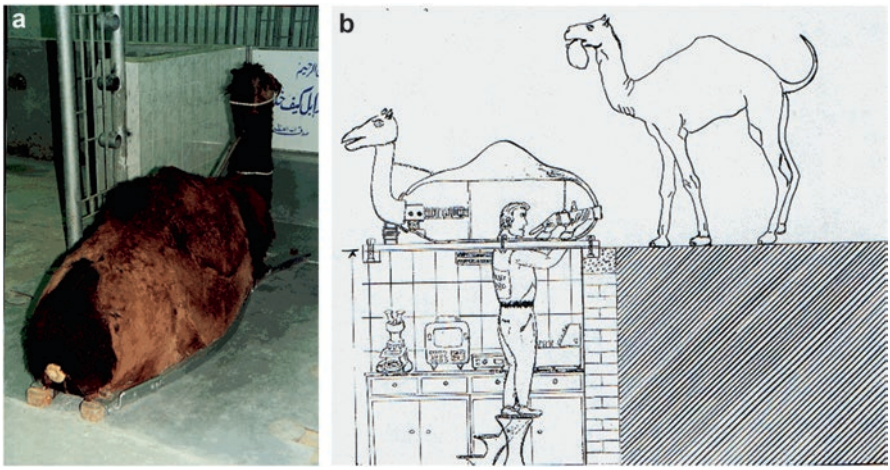

Fig. 2.19 Semen collection of male dromedary camel bull using a dummy and mounted artificial vagina with operator stays in a small laboratory underneath the dummy. (**a**) A hind view for the dummy to indicate its external feature and the mounted AV, (**b**) A diagrammatic drawing for the dummy and the small lab underneath. (Photo and diagram reprinted with permission of IVIS editor from El-Hussanein 2003)

2.6.4 Initial Sperm Examination

The available studies on the ejaculate initial quality of camel sperm showed high variability of the camel sperm characteristics.

2.6.4.1 Macroscopic Examinations

Volume

The sperm volume of the dromedary varies considerably depending on the technique and means of collection as well as the male's libido and the time of stimulation before collection. Using electro-ejaculator the volume varies from 1 ml to 10 ml (Tibary and Anouassi 1997b) even this volume varies from 2 ml to 12.6 ml when semen collection is carried out using AV (Wani et al. 2008; Tibary and Anouassi 1997b). According to Monaco et al. (2018); more than four ejaculates per collection session of about 45 min is possible resulting in total ejaculation volume of 12–16 ml. Even though allowing time for the male to recover after each ejaculation, the concentration of sperm was slightly lower after the third ejaculate.

Fig. 2.20 Semen collection of male dromedary camel bull using candom device mounted within the vagina of receptive females and secured with a harness. (**a**) Condom device (left) inserted in the vaginal of an estrous female (right), (**b**) Female with secured intravaginal condom in the breeding pen (**c**) Male teasing the female with intravaginal condom (**d** and **e**) Male breeding within the intravaginal condom. (Photos reprinted with permission of the editor: Ahmed Bamouh, from Tibary and Anouassi 2018)

Appearance and Consistency

Semen of camels is of gray to milky white color (Bravo et al. 2000). Sand contamination can change semen color, for this reason the proposed camel dummy with inserted internal AV prevent semen contamination (El-Hussanein 2017; Skidmore 2019). Camel semen is in gel form and the proportion of seminal fluid varies leading to high variability of viscosity and sperm concentration (Deen et al. 2003). Depending on individual males, semen collection process, stimulation duration and the volume of the gel fraction in the ejaculate, this proportion of the seminal fluid is highly variable. (Tibary and Anouassi 2018; Skidmore et al. 2013).

The main constraint related to camel sperm viscosity is due to the distribution evenly throughout ejaculation of the liquid fraction which induces a difficulty of its separation (Skidmore et al. 2013). It was suggested that the high semen viscosity is resulted of higher seminal plasma levels of glycosaminoglycans or mucin 5B (Kershaw-Young et al. 2013).

It is important that the sperm of the dromedary undergoes a total liquefaction before its microscopic examination and dilution. Tibary and Anouassi (1997b) reported that sperm with little seminal fluid liquefies faster than sperm that contains large volume of seminal fluid. In addition, some studies have shown that liquefaction time of the camel sperm at 25–38 °C is 8–20 min (Tibary and Anouassi 1997b),

15–30 min at 30–37 °C (Al-Qarawi et al. 2002) and 90 min at 37 °C (Wani et al. 2008). However, during these time ranges, change in sperm viscosity also constitutes a partial liquefaction that is subject to a high variance between males and between ejaculates from the same male, ejaculate manipulation technique during incubation, type of extender and quantity of mucin in the ejaculate.(Tibary and Anouassi 2018; Al-Bulushi et al. 2018). The complete liquefaction of dromedary ejaculates took 8 h (Tibary and Anouassi 1997b). In recent study, this time was estimated to 23.9 ± 1.5 h (Mal et al. 2016). In practice, this time would certainly result in loss of quality of semen (Tibary and Anouassi 2018). Fast liquefaction (after 1.5 h at 37 °C) of camel diluted 1: 1 using Tris – lactose egg yolk, Tris – tes egg yolk,, sucrose egg yolk, citrate egg yolk and Tris – fructose egg yolk extenderscompared to semen without an added extender (Wani et al. 2008). Attempts at rapid liquefaction of dromedary sperm using mucolytic and proteolytic compounds such as plasmin, trypsin, chemotrypsin, amylase, collagenase and ficin have given variable results (Tibary and Anouassi 1997b; Bravo et al. 2000; Ghoneim et al. 2010; El-Bahrawy et al. 2015; Monaco et al. 2016; Keshavarz et al. 2016). Results using collagenase (0.5–1%) or papain (1.7 Unit/ml) seems to provide the best semen liquefaction in DC(20–40 min) (Kershaw-Young and Maxwell 2012; Tibary and Anouassi 2018; Monaco et al. 2016). These enzymatic treatments improve post-thaw motility and velocity patterns of spermatozoa but also can be deleterious to spermatozoa (Rateb et al. 2019; Skidmore 2019). Mechanical processes were also investigated to improve the duration of semen liquefaction using prolonged method of dilution (1: 1–1: 5) (Wani et al. 2008; Malo et al. 2017) and application of ultrasound (Rateb 2016; Skidmore 2019). The camel semen treatment by amylase improves viscosity score and mobility. The association of this enzymatic treatment with centrifugation for seminal plasma removal decreases semen viscosity and motility but increases the rate of spermatozoa abnormalities (El-Bahrawy 2017a, b).

2.6.4.2 Microscopic Examination of Semen

After first dilution (1:1) using semen extender and complete semen liquefaction the microscopic evaluation of camel sperm can be performed by measuring concentration, total motility, individual motility, viability and morphological aspects (Table 2.4). After that the second dilution can be applied to final ratio (1: 3).

Table 2.4 Mean characteristics of camel semen collected by the artificial vagina (Bravo et al. 2000)

Volume (ml)	Concentration	Motility (%)	Stained dead sperm (%)	Sperm showing morphological abnormalities (%)	Sperm showing acrosome abnormalities (%)
7.5	325	51	18	28	8.5

Concentration

The concentration expresses the number of spermatozoa per mm^3 (or per ml). It can be determined directly by counting spermatozoa using a hematimetric cell or indirectly by visual comparison of the sperm with standard solutions, by electronic counting or by nephelometry (spectrophotometer or colorimeter). This last method can however be indirectly increased following the presence of debris (sand) in the sperm of the dromedary and thus a variation of the opacity.

Direct counting is done using a hemacytometer. This type of count assumes the prior dilution of the sperm. The dilution rate depends on the apparent concentration of the sperm and the desired final concentration. There are different types of hemacytometer which are characterized in particular by their surface (S) and the depth of their counting chamber (P): example Thoma (S: 1 mm^2, P: 0.1 mm). The hemacytometer consists of a glass slide, hollowed out of a small cuvette whose bottom is lined with a grid. Thoma's cell has a grid of 16 large squares each comprising 16 small squares. The area of the large squares is equal to 1 mm^2. The counting chamber is 0.1 mm high.

After depositing a drop of diluted sperm and covering it with a coverslip, the number of spermatozoa is determined at magnification 400 on a surface corresponding to 4 large squares. Conventionly, only the heads of spermatozoa located inside the two parallel lines delimiting each large square or whose head is on the left and upper lines delimiting a large square are taken into account. The concentration is calculated as follows: Concentration = $N \times 4 \times 10 \times D$ (N is the number of sperm counted in 4 large squares, 4 since the hemacytometer has 16 large squares with a total area equal to 1 mm^2, 10 since the height of the enumeration chamber is equal to 0.1 mm and D is the degree of the initial dilution).

The sperm concentration and the total number per ejaculate of male DC varies among studies ranging from 80 to 1300×10^6/ml (Tibary and Anouassi 1997b; Wani et al. 2008; Al-Bulushi et al. 2018) and from 240 to 2576×10^6 (Tibary and Anouassi 1997b; Morton et al. 2011; Tibary et al. 2014; Tibary and Anouassi 2018). Camel semen concentration is qualified as oligozoospermia when it shows less than 40 millions spermatozoa/ml (Waheed et al. 2014). Specific individual variations, frequency of collection and technique/time needed for semen collection can lead to significant variation of sperm numbers in the dromedary ejaculate. (Tibary and Anouassi 2018; Al-Bulushi et al. 2018). It is also interesting that authors have mentioned that the absence of sperm in many ejaculates is due to incomplete ejaculation (Hassan et al. 1995). Weekly semen collection during the peak breeding season for 3 weeks or twice weekly semen collection over 8 weeks did not affect the semen quality (Al-Bulushi et al. 2018). The pregnancy rate using fresh camel sperm with low concentration (75×10^6 sperm/ml) showed significantly low rate compared to sperms with concentration of 150 and 300×10^6 sperm/ml (et al. 2019b).

Massal Motility

Sperm mobility is evaluated according to two criteria: mass and individual mobility. The mass mobility is evaluated on diluted sperm by placing a drop on a preheated slide and observes it under a phase contrast microscope in low light intensity at 100× magnification. It refers to the intensity of the waves caused by the movement of sperm. A score of 0 to 5 is assigned to the observed sample: 0: no movement of the sperm, 1: slight noticeable movement, no waves, 2: few waves, 3: many waves, 4: fast and intense waves and 5: very fast waves.

The nomenclature of asthenozoospermia was given to camel sperm with decreased spermatozoa motility (Waheed et al. 2014). In Al-Bulushi et al. (2019b) study it was showen that insemination using raw camel sperm with 84–86% total motility result on high and comparable pregnancy rate (81%) to that obtained by natural mating (83%).

Individual Mobility

Individual mobility is assessed on a drop of diluted and sufficiently liquefied sperm placed on a preheated slide under ×400 magnification. The dilution is done in a diluter ("extender") previously heated to a ratio of 1 to 20 (0.25 ml of sperm and 4.75 ml of diluter). These media should be ideally prepared before the examination to avoid any change in pH, detrimental to the motility of the spermatozoa. Three to five fields close to the center of the drop will thus be examined and the average calculated. The individual motility is the proportion (%) of sperm that pass through the field of the microscope relatively quickly with rotational movements of the head (flexing or tracer sperm). Some sperm spinning in small circles or moving backwards is due to the abaxial implantation of their tail. Others move in a curvilinear fashion or more slowly. They are not considered mobile. Computer Assisted Sperm Analysis (CASA) type image analyzers make it possible to quantify more precisely (the nature and speed of movements).

A very good quality sperm must have at least 50% of motile spermatozoa in the camel (Bravo et al. 2000). Numerous reports from the literature have not revealed sperm mobility problems of dromedary sperm, however, we find that the proportion of motile sperm varies from 0 to 80% (Tibary and Anouassi 1997b; Deen et al. 2003) and that is significantly influenced by several factors such as means of collection, technique and conditions of collection, sperm viscosity, nature of the diluter, the synthetic material of the inner lining of the AV and the length of time that the sperm spends inside AV (Tibary and Anouassi 1997b; Deen et al. 2003). Addition of caffeine could improve the individual motility of the camel sperm (Deen et al. 2003).

The examination of individual motility is interesting because it indirectly provides information on the integrity of the sperm membrane and its morphological integrity. Thus, a high percentage of motile sperm combined with a high percentage of dead sperm suggests improper handling of sperm rather than abnormal sperm.

Likewise, low motility is often correlated with a high percentage of abnormal forms or dead sperm.

Morphological Examination

Morphological examination requires staining of the sperm by placing a drop of semen on the end of a slide and spreading it in a thin layer with another blade tilted at 45°. The preparation is dried in air for few minutes. The smear is then fixed by immersion in a solution of methyl alcohol or in a solution of 5% formalin. Free air fixation is also possible such as passing the smear quickly over an alcohol lamp. Any thermal shock will nevertheless be avoided during these preparations. The preliminary dilution of the sperm (2 drops in 0.5 ml of diluent) will facilitate the morphological examination. Some stains are intended to better show the morphology of the spermatozoon (total staining), the others called vital make possible to differentiate between dead and living spermatozoa. Among the total stains, some are said to be simple (India ink, methylene blue, toluidine blue, gentian violet, fuschin, etc.): they provide uniform coloration of the spermatozoa while the second called double (Giemsa, Williams) and better show the structural differences at the level of the head, acrosome or intermediate piece. The principle of vital staining is to use a stain which only crosses the membranes of dead cells (eosin, rose bengal, cresyl green) and a background stain which facilitates reading (methylene blue, nigrosine). Eosin-nigrosin staining is conventionally used with a sperm-stain mixture of between 1/10 and 1/20.

The examination will ideally be carried out with an immersion objective at ×1000 magnification. One hundred spermatozoa will be counted or even more if the number of sperm is greater. Dead sperm will be stained red due to eosin having entered owing to changes in membrane permeability.

Sperm morphological abnormalities can be said to be primary (1) if they originate during the spermatogenesis phase (testis) or secondary (2) if they occur during their maturation phase (epididymis). The majority of sperm lesions are said to be primary. Some can be both primary and secondary such as the presence of droplets, tailless heads. Sperm abnormalities can also be classified as major or minor depending on whether or not they have a negative effect on fertility. Finally, they can affect the various parts of the spermatozoon individually or simultaneously.

The head may show abnormalities in the shape, size, duplication, position or structure of the acrosome. The anomalies of the intermediate piece concerned cytoplasmic rest and angulation. The tail can be too short, too long, absent, duplicated, coiled at the end of the flagellum, or even under the head, and even around it or angulated (angulation exceeding 90°).

A rate of 10% of abnormal spermatozoa has been reported in camel sperm (Al-Bulushi et al. 2018) without significant effect of the frequency of collection on this parameter. The teratozoospermia of camel sperm was given to ejaculates with more than 40% abnormal spermatozoa (Waheed et al. 2014). The proportion of

protoplasmic droplet spermatozoa was 1.02 ± 0.2 on average, while 2.7 ± 0.6 and 9.7 ± 2.9% had mid-piece and tail defects, respectively (Wani et al. 2008).

2.6.5 Sperm Dilution and Conservation

2.6.5.1 Fresh Semen Dilution

The purpose of camel sperm dilution is to increase the total mass of the sperm fraction to split the ejaculate into fertilizing doses while adding substances that ensure sperm survival during storage. The latter can last a few hours or from a few months to several years.

In the dromedary, the separation of the liquid fraction from the sperm is difficult due to the distribution evenly throughout ejaculation of the liquid fraction. In addition, the sperm consists of a very variable volume of glandular secretions and that the testicular secretions constitute the essential of the semen.

The dilution medium requires the following characteristics: osmotic pressure which must be isotonic with the sperm and be able to maintain it during the storage period, contains colloidal substances (egg yolk, lipoproteins, lecithins) to ensure spermatozoa protection, contains buffer substance which makes possible to maintain a pH favorable to spermatozoa but its presence is not too important given the low concentration of spermatozoa in the sperm of the dromedary leading to a slight decrease in pH, contains nutrients to support metabolism, vitality and longevity of spermatozoa and contains antimicrobial agents.

The well diluted sperm offer best condition to spermatozoa and allow them to fulfill their 4 functions prior to fertilization: metabolic activity producing energy, mobility to progress in the female genital tract, protective enzymes on the acrosome to facilitate penetration into the oocyte and the presence of proteins on the plasma membrane to ensure their optimal survival in the female genital tract and their attachment to the pellucid of the oocyte.

To use camel sperm few minutes to a few hours after collection, it must be diluted at a ratio of 1:1 to 1:3 in a suitable diluter previously warmed. The dilution depends on the concentration of the ejaculate and has objective to achieve a standard concentration of 100×10^6 sperms/ml (Anouassi et al. 1992). This value can be revised downwards or upwards depending on the quality of collected sperm and insemination method (Bravo et al. 2000; Skidmore and Billah 2006a). Diluted sperm can be stored at room temperature (37 °C) until insemination (Tibary and Anouassi 1997b).

2.6.5.2 Short Term Preservation

If the sperm is stored for up to 24 h, it is mandatory to gently decrease the temperature of the mixture sperm-diluter to 4 °C by placing the tube in a water bath at room temperature and then refrigeration to reach 5 °C after about an hour (Tibary and

Anouassi 1997b). Equitainer of equine semen can be used for the semen transport during 18–36 h. The researches on camelid semen extenders remind scarce. Most extenders used for camel sperm are adapted from bull and stallion commercial sperm extenders as Glucose-EDTA, Dimitropolous 11, INRA-96, Sodium-citrate egg yolk, Skim milk, Kenny's equine extender, lactose egg yolk (Tibary and Anouassi 1997b, 2018) and have showed a low impact on sperm motility and viability. A commercial extender has been available for camel semen since the early 90s (Camel Buffer Green®, IMV, L'aigle, France). Recently, Al-Bulushi et al. (2019a) recorded that INRA is less suitable than Green Buffer to preserve semen at refrigeration for 24/48 h. It is mandatory to remember that for short-term preservation of camel sperm, it is essential to leave the time necessary for the complete liquefaction of the sperm before adding the diluter in order to obtain a homogeneous mixture (Tibary and Anouassi 1997b) and possibly reduce the effects of dilution on sperm mobility. It will be remembered that there are still great variations in the characteristics of the sperm of dromedaries subjected to short-term storage.

2.6.5.3 Long Term Preservation

If semen is to be used beyond 24 h after collection, freezing should be considered. There is no consensus on successful strategies for maintaining the fertilizing lifetime of frozen-thawed camel spermatozoa. The large scale application of AI in dromedary camels is impeded by this constraint. Freezing requires use of cryoprotectant agents. In view of the potential deleterious effects of cryoprotective agents on the sperm, they must be used at an optimal dilution which allows their benefits to be obtained and prevent osmotic and thermal shock from occurring both during dilution, cooling, freezing and thawing processing (Watson 2000).

Semen from DC was frozen successfully in a variety of extenders: Green Buffer® Egg yolk-glycerol, INRA and Egg yolk (Crichton et al. 2015), Tris-egg yolk-glycerol (Deen and Sahani 2006). Two steps are required for the cryopreservation of dromedary spermatozoa: dilution with cooling extender (80 ml Lactose 11%, 20 ml Egg Yolk) and dilution with freezing extender (95.5 m cooling extender, 06.0 ml Glycerol and 1.5 ml Orvus paste –Equex). Immediately after collection, the cooling extender is added to the semen. The freezing extender is characterized by the fact that it contains a 7% of glycerol and an Orvus paste (emulsifying agent) that plays a role in plasma membrane stabilization of the sperm.(Tibary and Anouassi 2018). The first diluter is maintained at 32 °C and the second diluter at 4 °C. The Tris based extender with 7% glycerol and sucrose based extender (73.0 ml Sucrose 12%) showed superiority to preserve post-thaw spermatozoa quality (mobility and acrosome integrity) and fertility of camel bull (Zhao et al. 1994; Tibary and Anouassi 2018; Akbar et al. 2018). Likewise cholesterol-loaded cyclodextrin enhanced cryosurvival, post thaw sperm motility (0 h and 3 h post thaw) at room temperature and post-thaw sperm progressive status (Crichton et al. 2015, 2016).

Packaging sperm in pellets is rarely practiced in dromedary camels. Pellets have the disadvantage of not being able to be identified correctly. More conventionally,

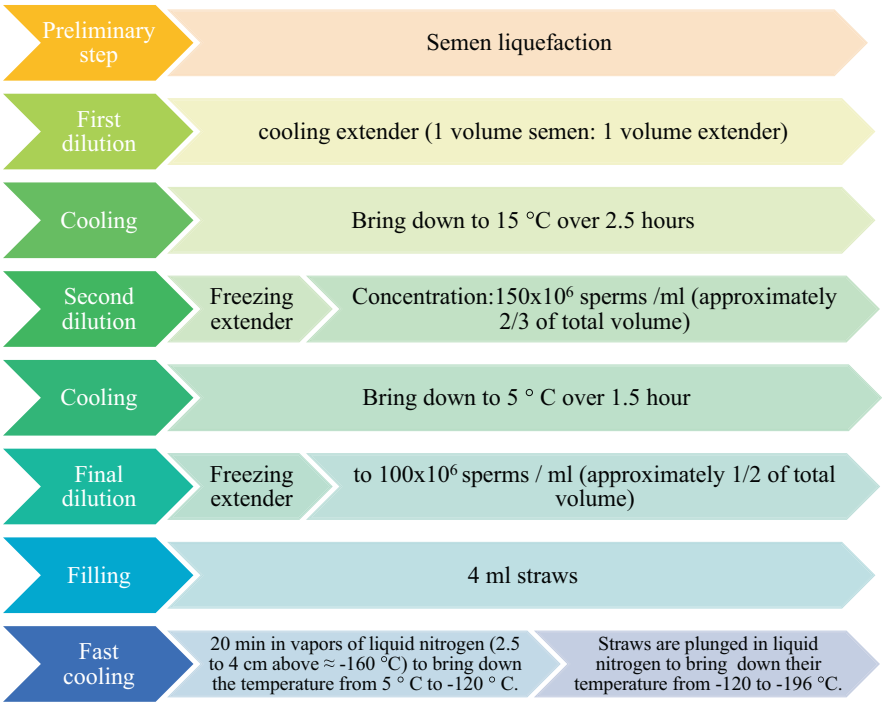

Fig. 2.21 Steps of camel sperm freezing in 4 ml straws

the sperm is packaged in polypropylene or polyvinyl straws with volumes between 0.25 and 4 ml. The required sperm dose has typically been a 4 ml straw that will contain 100×10^6 sperms/ml (Tibary and Anouassi 2018).

After semen liquefaction, freezing process depends mainly on the volume of the straws. When using large straws (4 ml) we proceed as follows (Fig. 2.21):

When we use semen package of 0.25 or 0.5 ml straws, the freezing process starts after total liquefaction according to the following steps (Tibary 2001; Deen et al. 2003; Niasari-Naslaji et al. 2006, 2007; El-Bahrawy 2010; Crichton et al. 2015):

- Dilution and cooling to 5 °C over 1 h.
- Semen package and equilibration at 5 °C for 2 h.
- Fast cooling by straws exposure to liquid nitrogen (4 cm above liquid nitrogen level) for 10 min
- Transfer of the cooled straws directly into liquid nitrogen.

Recent study performed at Camel Reproduction Centre, (Dubai, United Arab Emirates) by Malo et al. (2020), showed that camel sperm quality (total motility, viability and acrosome status) did not change significantly after 24 h cooling at 4 °C using two extenders (INRA 96 and Green Buffer) with or without cryoprotectant (3% glycerol or 3% ethylene glycol). Same authors reported also that shorter equilibration times (0–60 min) did not affect results of post-thaw sperms although

Glycerol 3% and ethylene glycol provided the best motility recovery rates compared to methyl formamide, dimethyl sulfoxide (Malo et al. 2017, 2020). In Addition, the post thawing results using Green Buffer showed highest rates of total and progressive motility and viability (Malo et al. 2020). For sperm viability, INRA with Ethylene Glycol 3% showed the lower rates compared to other extenders-cryoprotectant mixture (Green Buffer with glycerol 3% or Ethylene Glycol 3% and INRA 96with glycerol 3%) or buffers without any cryoprotectants (Green Buffer and INRA 96). Al-Bulushi et al. (2019a, b) recorded that better semen quality was observed using Triladyl (containing glycerol as cryoprotectant). In addition, the addition of catalase to extenders can act during prolonged sperm handling to prevent oxidative damage and thus promote chilled and cryopreserved sperm to retain better motility characteristics (Medan et al. 2008; Malo et al. 2019, 2020).

2.6.6 Sperm Thawing

Different thawing speeds were recommended depending on the packaging used for camel sperm.

The content of pellets is thawed out by dropping them into heated receptacles or incorporate it to thawed extender (Tibary and Anouassi 2018). Semen frozen in ampules is thawed out by placing them for 30 s to 1 min in a water bath set at 45–55 °C (Bravo et al. 2000). Straws (0.25 or 0.5 ml) can be thawed by placing them in water at 40 °C for 8 s or 30–60 s if the water is at 35–37 °C. The 4 ml straws are thawed at 50 °C for 40 s by continuous agitation in the water bath (Tibary and Anouassi 2018; Bravo et al. 2000). Cryovials are thawed out for 2 min by immersion in a water bath at 40 °C (Deen et al. 2003).

In recent study, Malo et al. (2020) reported that sperm extended in Green Buffer maintained better post-thaw vitality than sperm in INRA 96. The analyze of the effect the interaction of extenders with cryoprotectants on sperm vitality showed that INRA96-ethylen glycol 3% exerted most adverse effect on sperm vitality compared to INRA96-Glycerol 3%, Green Buffer-ethylene glycol 3%, Green Buffer-Glycerol 3% and both extenders INRA96 and Green Buffer without cryoprotectants. In other hand, all aforementioned sperm treatments did not showed any post-thaw effect on acrosome integrity (Malo et al. 2020).

Liquefaction of camel semen using Tris lactose supplemented with mucolytic agent (amylase) followed by centrifugation of seminal plasma removal in the presence of antioxidant agent (caffeine) improved significantly post-thaw motility and recovery rate suggesting a particular interest of this refined protocol for camel semen cryopreservation (El-Bahrawy 2017a, b). Similarly, semen extender supplemented with trace elements zinc and selenium nanoparticles (ZnONPs and SeNPs) improved significantly camel sperm vitality, progressive motility, ultrastructural morphology, sperm membrane integrity and decreased apoptosis when frozen and thawed (Shahin et al. 2020). These results meet the previous established conclusion about advantageous effects of nanotechnology in sperm function by incresing

antioxidant effect and bioavailability and reducing the undesired liberation of toxic concentrations (Falchi et al. 2018; Shahin et al. 2020).

Damage that occurs during freezing-thawing processes primarily affects cell membranes (plasma and mitochondrial) and, in more serious cases, the nucleus (Ahmed et al. 2017). Evaluating the percentage of motile spermatozoa at various intervals during incubation is a classic method of evaluating the method of freezing/thawing semen. The development of computerized mobility analysis systems helped to reduce the subjectivity part of this assessment. (Tibary and Anouassi 2018) reported other evaluation methods proposed for camel species such as hypoosmotic swelling test (incubation in fructose or sucrose solution of 50–100 mOsm at 37 °C for 45 min), special staining techniques (Isothiocynate-conjugated peanut agglutinin) (Morton et al. 2007, 2008), Chlortetracycline staining for spontaneous capacitation (Crichton et al. 2015). The correlation between all these methods and the pregnancy rate remains to be demonstrated after a wide application of AI in camel herds.

2.6.7 Insemination Technique

Compared to other animals such as saw and ewe, camel insemination is simpler since the cervix is shorter and straighter and the uterus is less coiled, so it is easier to pass a catheter through the cervix and to direct it up the uterine horn per rectum (Skidmore et al. 2013). The ovarian function of the female camels must be monitored by series of ultrasonography exams to follow the growth of the dominant follicle which will be ovulated before AI. Ovulation induction is commonly performed out with a single injection of GnRH/hCG given intravenously when there is a 1.3–1.7 cm antral follicle in the ovaries (Skidmore et al. 1996; Medan et al. 2008). AI must be carried out 24 h after the injection of GnRH/hCG. The equipment consists of an insemination gun of 40–45 cm length and 5–6 mm diameter with an outer body and an inner mandrel. It is completed by an external plastic sheath attached to the insemination gun by means of a small washer. The fresh or thawed semen straw is wiped to remove all traces of water (water = spermicide) and the identity of the camel bull is immediately verified. It is then sectioned approximately 1 cm from its closed end and introduced into the insemination gun previously heated by friction to avoid any thermal shock. A plastic sheath (disposable) ensures the sanitary protection and the tightness of the device. It is possible to fit a flexible catheter within the rigid outer catheter preheated to 38 °C to avoid thermal shock for deep uterine insemination (Fig. 2.22) (Tibary and Anouassi 1997c).

The insemination technique is that of cervical catheterization with immobilization of the latter rectally (Fig. 2.22). The right or left hand introduced into the rectum, grasps the cervix and the other hand introduces the catheter into the vulva (previously cleaned), pushing it forward and following the ceiling of the vagina (angle of 45°) to avoid the urinary meatus. Vaginal folds are avoided by pushing the cervix held with the right or left hand forward (this clears the folds).

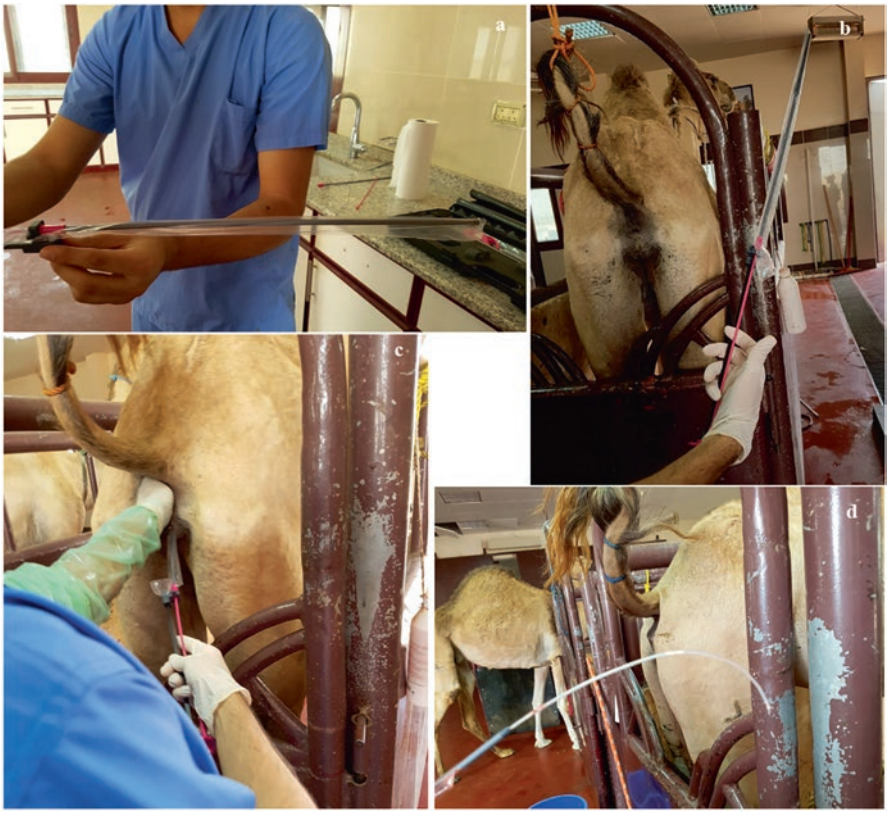

Fig. 2.22 Artificial insemination technique using special catheter for deep uterine deposit of camel semen. (**a**) Sterile deep horn insemination gun (**b**) Female prepared for deep horn insemination (**c**) Insertion of the deep horn insemination gun and transrectal guidance of the flexible catheter (**d**) Deep horn AI gun after insemination. Note the flexible catheter within the rigid outer catheter. (Photos reprinted with permission of the editor: Ahmed Bamouh, from Tibary and Anouassi 2018)

Locating the orifice of the cervix into which the catheter tip is to enter requires skill and must be done without any brutality. The hand that moves the cervix must, by the way a glove is put on, manipulate the cervix so that it comes over the catheter while avoiding the cervical folds until it reaches the cervico-uterine portion. Although it is easy to pass the catheter through the open cervix of the female during oetrus, under no circumstances should the catheter be forcefully pushed into the cervix (risk of injury).

It was therefore suggested that the semen should be deposited at the tip of the ipsilateral uterine horn to the ovary showing the mature follicle, thus closer to the uterine tubule junction rather than the uterine body (Skidmore 2019; Al-Bulushi et al. 2019b). The index finger of the hand inserted into the rectum controls, through the rectal wall, the correct position which allows the semen to be deposited (push

Table 2.5 Pregnancy rate of female camel according to different methodologies of artificial insemination

Author	Ovulation induction	Insemination time	Type of semen		Collection method	Females inseminated	Place of deposition	Pregnancy rate (%)
Deen et al. (2003)	hCG	24 h post hCG	WF	300×10^6	AV	13	UH	40
	hCG	48 h post hCG	EC	300×10^6	AV	10	UH	0
	hCG	24post hCG	FT	300×10^6	AV	10	UH	7
Medan et al. (2008)	hCG	48 h post hCG	WF	100×10^6	AV	13	UB	46
		48 h post hCG	EC (free-catalase)	100×10^6	AV	9	UB	22
		48 h post hCG	EC (with catalase)	100×10^6	AV	8	UB	37
Anouassi et al. (1992)	Vasectomized male		WF	100×10^6	AV		UB	80
McKinnon et al. (1994)	hCG		WF		AV			32
Skidmore and Billah (2006a)	GnRH		E	150×10^6	AV	15	UB	53
	GnRH		E	150×10^6	AV	14	UH	43
	GnRH		E	80×10^6	AV	14	UB	7
	GnRH		E	80×10^6	AV	15	UH	40
	GnRH		E	40×10^6	AV	14	UB	0
	GnRH		E	40×10^6	AV	14	UH	7
Al-Bulushi et al. (2019b)	GnRH	Immediately after ovulation treatment	WF (3–8 ml)	474×10^6	AV	11	UB	81
	GnRH	Immediately after ovulation treatment	WF (1.5–3.5 ml)	442×10^6	AV	10	UB	60
	GnRH	Immediately after ovulation treatment	WF (1 ml)	454×10^6	AV	12	UB	33

(continued)

Table 2.5 (continued)

Author	Ovulation induction	Insemination time	Type of semen		Collection method	Females inseminated	Place of deposition	Pregnancy rate (%)
Al-Bulushi et al. (2019b)	GnRH	Immediately after ovulation treatment	E	300×10^6	AV	17	UB	35
	GnRH	Immediately after ovulation treatment	E	301×10^6	AV	18	UH	72
Al-Bulushi et al. (2019b)	GnRH	Immediately after ovulation treatment	E	302×10^6	AV	19	UH	68
	GnRH	24 h post GnRH	EC	303×10^6	AV	20	UH	70
	GnRH	30 h post GnRH	EC	304×10^6	AV	17	UH	23
Al-Bulushi et al. (2019b)	GnRH	24 h post GnRH	E (3.5 ml)	300×10^6	AV	20	UH	70
	GnRH	24 h post GnRH	E (1.7 ml)	150×10^6	AV	23	UH	65
	GnRH	24 h post GnRH	E (0.9 ml)	75×10^6	AV	22	UH	40
Al-Bulushi et al. (2019b)	GnRH	24 h post GnRH	EC (Triladyl)	300×10^6	AV	18	UH	11
	GnRH	24 h post GnRH	EC (Optixcell)	300×10^6	AV	17	UH	5
	GnRH	24 h post GnRH	EC (Triladyl)	500×10^6	AV	19	UH	21
	GnRH	24 h post GnRH	EC (Optixcell)	500×10^6	AV	16	UH	12
	GnRH	24 h post GnRH	FT	300×10^6	AV	23	UH	0
	GnRH	24 h post GnRH	FT	500×10^6	AV	20	UH	0
Morton et al. (2010)	GnRH	24-48 h post GnRH	FT	150×10^6	AV	11	UB	27
Akbar et al. (2018)	GnRH	30 h post GnRH	FT	100×10^6	AV	21	UB	42[a]

UH uterine horne, *UB* uterine body, [a]pregnancy at 90 days post insemination, *WF* whole fresh, *E* extended, *EC* extended cooled, *FT* frozen thawed, *AV* artificial vagina

exerted on the plunger) at just this optimal level. The utero-tubal zone acts as a reservoir for spermatozoa. These are released regularly and continuously in order to ensure fertilization in the upper third of the oviduct without there being polyspermia (Skidmore 2019).

2.6.8 Factors Affecting Fertility After AI

AI with raw undiluted camel ejaculates resulted in wide variability of pregnancy rate: 40–81.8% pregnancy rate (Table 2.5; Anouassi et al. 1992; Deen et al. 2003; Skidmore and Billah 2006a, b; Medan et al. 2008; Morton et al. 2010; Al-Bulushi et al. 2019b). These results are somewhat comparable to results of timed natural mating programs: 67–71.4% (Manjunatha et al. 2015, 2018; Al-Bulushi et al. 2019b). Low volume (1 ml) insemination with undiluted ejaculate resulted in lower pregnancy compared to whole and split undiluted ejaculate. (Al-Bulushi et al. 2019b). However, some studies showed lower or null results of pregnancy rate using chilled and non diluted semen for AI (El-Hussanein 2003; Medan et al. 2008; Morton et al. 2013).

The pregnancy rate after AI using chilled camel semen diluted by different extenders show large variability of pregnancy rate (Table 2.5): 0% using skimmed milk (Skidmore 2000); 5.9–12.5% using Optixcell (Al-Bulushi et al. 2019b); 11.1–21.1% using Triladyl (Al-Bulushi et al. 2019b); 17–34% using INRA 96 (Morton et al. 2010, 2013; Malo et al. 2020); 50% using lactose plus egg yolk (Anouassi et al. 1992), 53% using Laciphos (Skidmore 2000); 10–72.7% using Green buffer plus egg yolk (Al-Bulushi et al. 2019a, b; Skidmore and Billah 2006a; Morton et al. 2010, 2011, 2013; Malo et al. 2020). The wide variability of the pregnancy rate after AI by liquid chilled semen would be difficult to explain especially when considering that in vitro semen quality appears to be high.

The post-thaw frozen semen showed promising consistency, but this is not evidenced by the pregnancy outcomes to date. Single early pregnancy in DC has (1/13 animals inseminated) resulted from AI with cryopreserved spermatozoa (Deen et al. 2003). The use of glycerol as cryoprotectant with Green Buffer extender during chilling did not interfere with cryosurvival, but it may be toxic to the fertility of fresh chilled sperm leading to null pregnancy rate (Malo et al. 2020). After that, Morton et al. (2010) reported a pregnancy rate of about 27% in single ovulated female camels using frozen-thawed semen diluated in Green Buffer and fertilization rates of about 11.1% in multiple ovulating camels. The first successful encouraging results for large scale utilization of frozen semen insemination of dromedary camels was recently reported by Akbar et al. (2018) after a series of experiments on semen processing, cryopreservation and AI giving live birth of camel calves at Camel Breeding Center, Dubai, United Arab Emirates date 2018 (Akbar et al. 2018). In this experiment; 21 inseminated females by frozen -thawed semen with tris based extender and 7% glycerol gave the following results: On the 30th day of insemination, 15 (71.42%) females were found to be pregnant. Between the 18th and 30th

days, 6 she-camels faced embryonic mortality (Akbar et al. 2018) and pregnancy was confirmed in the remaining females at 60 and 90 days of insemination (09). At mid gestation, two females aborted and the remaining seven females (33.33%) were carried to full term. More investigations are needed to confirm the fertility rate using frozen thawed semen of eventually large scale application of AI as important step of assisted reproductive techniques in camelids.

The monitoring of the ovarian follicular wave patterns of the female camel by transrectal ultrasound examinations is mandatory for AI success. Ovulation is induced by a single intravenous injection of GnRH (ex: 20 mg buserelin) accompanied by timed artificial insemination once the dominant follicle reaches 1.3–1.8 cm (Skidmore and Billah 2006b). Female camels inseminated 24 h after ovulation induction resulted in higher pregnancy rate (53.7–70.0%) (Skidmore and Billah 2006b; Al-Bulushi et al. 2019a, b) compared to insemination performed at the time of ovulation induction or 30 h avert induction (68.4 and 23.5%, respectively) (Al-Bulushi et al. 2019a, b).

The results of the insemination into the uterine body or the tip of uterine horn ipsilateral to the ovary containing the dominant follicle showed respective increasing conception rate of 0, 7, 53% and 7, 40, 43% according to increased sperm concentration per milliliter of 40, 80 and 150 × 10^6 (Skidmore and Billah 2006a). Deposition of semen in the uterine body resulted in lower pregnancy rates compared to deposition in the tip of the horn (35.3% versus 72.2%) (Al-Bulushi et al. 2019a, b). AI with 75 × 10^6 motile spermatozoa resulted in lower pregnancy rates compared to 150 and 300 × 10^6 motile spermatozoa doses (40.9% versus 65.2 and 70.0%, respectively) and pregnancy rate without significant correlation with semen extenders (Green buffer, Optixcell or Triladyl) (Al-Bulushi et al. 2019a, b). Indeed, the use of insemination with small sperm concentration requires a deep uterine insemination technique. If semen is deposited only in the uterine body rather than at the tip of the horn a substantial loss of spermatozoa occurs due to the semen's backflow through the short and open cervix during the female camel's oestrus.

The short, open cervix that occurs during oestrus of the female camel would lead to considerably loss of spermatozoa, due to backflow of semen through the cervix, when the semen is deposited just into the body of the uterus rather than at the tip of the horn (Skidmore and Billah 2006a; Al-Bulushi et al. 2019a, b). Uterine manipulation during the intrauterine insemination may simulate penile stimulation by male camel or aide sperm transport by a greater release of uterine hormones involved in uterine contractility (Martinez et al. 2002).

Form the given studies; it appears that large scale utilization of AI in camels faces many technical difficulties particularly those relative to oestrus synchronization, preservation of camel sperm and low fertility of females inseminated with frozen semen. In addition, many basic concepts relevant to AI application are still not available, such as infectious disease screening, biosecurity controls, male management, training procedures and hygienic collection and processing of semen (Monaco and Lacalandra 2019).

2.7 Conclusion

Good reproductive performances allow obtaining a high numerical productivity of camel herds and consequently increasing the information available on the siblings and shorten the average generation interval. However, in traditional reproductive management, increasing reproductive efficiency of camel herds faces certain constraints such as long gestation period, short breeding season and traditional reproductive and herd management methods leading to widespread venereal infections and reducing fertility. Oestrus synchronization and AI are simplest and cheapest reproductive technologies to overcome some of these problems. They have a significant influence on the genetic improvement rate. A higher reproduction rate means a lower number of breeding animals for a given population size and, thus, a greater selection strength. A greater number of offspring per breeder also promotes a more accurate estimate of genetic values. Another advantage is the faster diffusion of higher genetic material and international movement of genetic material. To date, it is difficult to accept large scale practice of artificial insemination in DCbecause of the technical and zootechnical difficulties associated with this biotechnology. Investigations on the technical aspects are still in progress; such as semen collection methods, semen liquefaction and cooling, deep freezing and thawing of camel spermatozoa to optimize AI protocols and standardize procedures. The main zootechnical constraint is the nature of the breeding systems of camel herds which are in continuous movement throughout the year in pastoral areas. This makes difficult the ultrasound monitoring of ovarian activity, ovarian activity synchronization followed by timed ovulation induction and AI or blind mating. In addition, there is the risk of inbreeding and the reduction in reproductive capacities by using a reduced number of breeding males over a large number of females during short breeding season.

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