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Contents lists available at ScienceDirect

Asian Pacific Journal of Reproduction

journal homepage: www.apjr.netReview <http://dx.doi.org/10.1016/j.apjr.2016.01.003>

Multiple ovulation and embryo transfer (MOET) in camels: An overview

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ARTICLE INFO

Article history:

Received 2 Aug 2015

Received in revised form 16 Dec 2015

Accepted 27 Dec 2015

Available online 23 Jan 2016

Keywords:

Super stimulation
Multiple ovulation
Embryo transfer
Synchronization of oestrus
Camels

ABSTRACT

Unlike in other domestic animal species like cattle, reproductive biotechnologies like Artificial Insemination (AI) and Embryo Transfer (ET) are not well developed and thus are not being used as routine breeding procedures in camels. One of the important objectives of this manuscript is to focus on analyzing the present status of Multiple Ovulation and Embryo Transfer (MOET) in camels and its future perspectives. Camels are induced ovulators, thus require hormonal treatment to induce ovulation and control the follicular cycles, which is the main reason why protocols used in other domestic animal species cannot be directly used in this species. The review suggests that the best method for super stimulation of ovaries in camels is use of a combination of Equine Chorionic Gonadotropin (eCG) and Follicle Stimulating Hormone (FSH) at any stage after elimination of dominant follicle if any or at the early stage of the follicular wave and ovulation of the developed multiple follicles can be achieved by mating donors. The review highlights that a better pregnancy rate is achieved with recipients who ovulate 24 h after the donor.

1. Introduction

Reproductive biotechnologies like Artificial Insemination (AI) and Embryo Transfer (ET) are being utilized extensively to improve the production characteristics of various domestic animal species [1–4]; however, these techniques are not well developed and thus are not being used as routine breeding procedures in camels. Camels are induced ovulators, thus require hormonal treatment to induce ovulation and control the follicular cycles, which is the main reason why protocols used in other domestic animal species cannot be directly used in this species. In this overview, we have summarised the present status of MOET in camels and its future perspectives.

2. Selection and management of donors and recipients

The most important factor in the success of an embryo transfer program in any species is the quality and selection of the donor and recipients. A complete breeding soundness examination should be undertaken to select reproductively sound recipients for embryo transfer programme [1]. All the donor and

recipient camels should be subjected to a thorough health screening and breeding soundness examination before inducing into the programme. Screening for reproductive problems and other major contagious diseases like trypanosomiasis, brucellosis and camel pox is recommended in camels [5–7]. Ideally all potential recipient camels should be 5–12 years old, in good condition, free of parasites and diseases and have had at least one normal pregnancy and parturition [5]. Breeding soundness examination includes per rectal palpation and ultrasonography of the genital organs, vaginal examination and uterine cytology and culture if indicated [5,7,8]. Ovario-bursal adhesion is a common reproductive problem seen in dromedary camels [9] which will adversely affect the results of embryo collection and transfer (Binoy *et al.*, unpublished observations). Diagnosis and treatment of reproductive problems in donors before starting super ovulation programme is highly warranted. High pregnancy and calving rate up to 75% could be achieved on transfer of hatched blastocysts in recipients used after intensive screening procedures when compared with very low pregnancy rate of 22% obtained from recipients used without proper screening from an organized farm (Binoy *et al.*, unpublished data).

3. Super-stimulation of donors

Overall, super stimulation and ovulation in mammals are considered a staggering challenge in embryo transfer

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Peer review under responsibility of Hainan Medical College.

programme. In cattle one of the most important problems in super stimulation is the high incidence of non-responsive females, which fail to produce follicles and then there is individual variation in response as well [1,10]. This is the major problem experienced in super stimulation and ovulation in camels too [7,11,12]. Because of no reliable protocols for cryopreservation of camel embryo and also high variation in response to super stimulation, embryo collection after single ovulation is also a suggested methodology especially in certain situations like donors that are refractory to gonadotropin treatment or in case of limited recipient availability [7,12].

The major gonadotropic hormones used for super stimulation of ovaries in cattle like Follicle Stimulating Hormone (FSH) and Equine Chorionic Gonadotropins (eCG) or Pregnant Mare's Serum Gonadotropin (PMSG) have been successfully used by many workers in camels with slight modifications of dosage [7]. eCG at various doses ranging between 1 500 and 6000 IU and pFSH (400 mg) [13] or 1–3 mg ovine OFSH for 3–6 days [14] have been successfully used in super stimulation of camels. However, a combination of both eCG and FSH has produced better stimulation [15,16].

In primary studies, McKinnon and Tinson [14] did progesterone priming of donors with 100 mg of progesterone-in-oil (i.m.) daily for 10–15 days prior to super-ovulation treatment, which included either a single injection of 3 000–6 000 I.U. eCG or twice daily injections of 1–3 mg ovine FSH over 3–5 days. More embryos were recovered from those treated with FSH than from those treated with eCG by these authors. Later Vyas *et al.* [17] induced ovulation in the donor animal using human chorionic gonadotrophin (hCG; Day 0) and then administered either 50 mg (FSH-P) in 10 diminishing doses at 12 h intervals starting from Day 6 after hCG administration; or 75 NIH-FSH-SI units in eight equal doses at 12 h intervals starting from Day 8 after hCG administration. These authors reported a considerable individual variation in response between animals, but no significant difference between the two groups in terms of the super ovulatory response and number of embryos recovered.

Better results have been achieved by our group after protocols using both eCG and FSH. We induced ovulation in all donor animals with GnRH, then starting on Day 4 after ovulation a combination of 2 500 I.U. eCG (day 1) and 400 mg FSH (pFSH: Follitrophin), given in declining doses over a period of four days (days 1–4) starting from day 1. Donors were then screened and mated approximately 10 days later when the majority of follicles had reached a mature size of 1.3–1.7 cm in diameter. We have observed the mean \pm SEM number of follicle development, ovulation, total embryo recovery and useable quality embryo recovery of (12.75 \pm 2.93), (9.13 \pm 3.64), (8.50 \pm 3.27) and (6.25 \pm 2.47), respectively.

Even though various super-ovulation agents and protocols have been used with varying success rates, just like in other species, a wide individual variation in super ovulatory response have been noticed in camels too. Usually in regularly cycling cattle and other spontaneous ovulating species the ovulation of developed follicles after super stimulation is achieved by lyses of the corpus luteum by prostaglandin injection [18] or creating a hormonal mechanism suitable for spontaneous ovulation in some other instances [19]. In camels, however, mating induces ovulation [20] and hence this process is not important and super stimulation of ovaries can be initiated by injecting gonadotropic hormones literally at any stage after elimination of the dominant follicle or at the early stage of the follicular

wave and ovulation of the developed multiple follicles can be achieved by mating donors. However, the dominant follicle suppresses the subordinate follicles in the same wave [21]. Also, follicles present at the start of treatment tend to develop into overlarge follicles before the new stimulated wave of follicles have had a chance to develop [22]. Hence the best results, (i.e. the best stimulation of the ovaries) occur if the camel is treated with exogenous gonadotrophins when there is minimum follicular activity in the ovaries [23]. In addition, another approach is to use the ovulation inducing hormones like GnRH or hCG at the time of first mating to make sure the ovulation of maximum number of super-stimulated follicles takes place.

4. Synchronization of recipients

Close synchrony of the embryo stage with recipients is the next most important factor for the success of any embryo transfer programme. In camels best pregnancy rates are obtained in recipients that are negatively synchronized with the donor by 1–2 days [13,15]. Pregnancy rates dramatically decrease if the level of synchrony is increased to +1 or –3 days [15]. Hatched blastocysts collected on day 7 after ovulation (day 8 after mating) transferred in recipients within the accepted limit of asynchrony of \pm 1 day yielded an overall conception rate of 63% in dromedary camels (Binoy *et al.*, unpublished data).

Recipients can be selected on the basis of their follicular size from a large pool of animals and induced to ovulate with GnRH or hCG [5,24]. Two GnRH injections 14 days apart or two GnRH injections 14 days apart and PG on day 7 after the first GnRH were the most effective methods to synchronize ovulation in dromedary camels to a fixed time interval of 14 days after treatment [25].

5. Embryo recovery and transfer

There are various factors affecting embryo recovery rate. These may include super stimulation treatment, fertility of the donor and male, collection time after ovulation and technical expertise [1,5,13]. As in other species, surgical and non-surgical methods of embryo collection have been described in camels, but most widely applied method is non surgical approach. Embryo collection and transfer can be performed by controlling camel in sternal recumbency or in stocks while standing [7,12]. In camels, embryos are recovered on day 7 after ovulation (eight days after the first mating) by trans-cervical uterine lavage with varying success rates [13,15].

Non-surgical method of embryo collection and transfer need passage of catheter in to the uterus through cervical canal, which is achieved either by rectal manipulation of the genitalia or by directing the catheter in to the cervix through vagina. Usually flushing catheter of 18–22 gauges is used and flushing is done by filling uterus with embryo flushing media and recovering the media by gravity flow. Individual horn flushing or flushing of the entire uterus by fixing balloon just in front of the cervix are the two methods followed. Around 800–1 000 mL of flushing media is used for flushing one animal [7].

Screening of the flushed media after filtering and handling of embryos in camels are done by any of the methods standardized for other species like cattle [1]. Each embryo has to be evaluated and graded according to the stage and morphology of the

embryo, which can be classified as transferable (hatched blastocysts with normal appearance) and non-transferable embryos [5,22].

6. Conclusion

From the literature cited, it seems that till date best method for super stimulation of ovaries in camels is use of a combination of eCG and FSH. A better pregnancy rate is achieved with recipients who ovulate 24 h after the donor. The recipients can be prepared from a group of animals with follicle (13–17 mm) which are injected with GnRH or hCG 24 h after mating the donor. Embryos are flushed on day 7 after ovulation and transferred in recipients on day 6 after ovulation.

Conflict of interest statement

We declare that we have no conflict of interest.

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