Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(4): 480-484

Received: 11-05-2018
Accepted: 18-06-2018

Abhishek Kumar
PhD Scholar, Division of Animal Reproduction, Indian Veterinary Research Institute, Utter Pradesh, India

Balamurugan B
PhD Scholar, Division of Animal Reproduction, Indian Veterinary Research Institute, Utter Pradesh, India

Ajaz Ali
PhD Scholar, Division of Animal Reproduction, Indian Veterinary Research Institute, Utter Pradesh, India

Ramamoorthy M
MVSc. Scholar, Division of Animal Reproduction, Indian Veterinary Research Institute, Utter Pradesh, India

Desh Deepak
PhD Scholar, Division of Veterinary Medicine, Indian Veterinary Research Institute, Utter Pradesh, India

Correspondence
Abhishek Kumar
PhD Scholar, Division of Animal Reproduction, Indian Veterinary Research Institute, Utter Pradesh, India

Therapeutic approach to bovine reproductive disorders: Recent advances and future prospects

Abhishek Kumar, Balamurugan B, Ajaz Ali, Ramamoorthy M and Desh Deepak

Abstract
Reproductive disorders are the major threats antagonizing fertility as well as productivity of dairy cattle. In India, the likelihood of infertility problems increases especially in lactating cows and buffaloes which are unnecessary exploited to meet heavy demands of milk in the face of poor management practices. The objective of this present review was to highlight the therapeutic aspects such as nanotherapy, stem-cell based therapy and gene therapy which are uncommon and the advancements in these areas along with hormonal therapy for the alleviation of reproductive problems in dairy animals. Though management conditions like housing (surrounding environment), feeding and watering largely play an important role in the prevention of reproductive diseases, these are commonly alleviated through the use of antibiotics, herbal remedy, homeopathy and immunotherapy (immune modulators). Apart from this, there is need for advancement in hormonal therapy to make it more convenient for easy application without causing adverse effects in animal body. Nanotherapy, stem-cell based therapy and gene therapy have the potential which need to be explored for more targeted and specific treatment as well as for their future applications on large scale in the livestock sector.

Keywords: Hormonal therapy, Nanotherapy, Stem-cell therapy, gene therapy, dairy animals

1. Introduction
Indian dairy is an epitome of rural industry where marginal, small and medium dairy producers contribute to the national milk pail that is collected, pasteurized, pocketed and marketed by a multitude of government, cooperatives, private chilling and processing units [1]. Under these production settings, it is difficult, if not impossible, to ascertain the prevalence of infertility with its causes in the cow due to lack of records and deregulated movement of animals among the clients. The likelihood of infertility problems increases especially in lactating cows and buffaloes which are unnecessary exploited to meet heavy demands of milk under poor management practices. Heterogeneity in the production potential of the breeds, seasonal variations in feed and fodder availability and lack of uniformity in climatic condition at different regions prohibit the development of uniform strategy either management or therapeutics for the infertility problems [2]. As a consequence, addressing even the nutritional, management and other production related issues of infertility is largely on the basis of individual cow therapy rather than herd medicine which reduces the profit margin of dairy producer by increasing the veterinary costs [3]. It became interesting when one considers no change in incidence of reproductive problems in dairy animals especially in India in spite of major advancements till date. Some of the most common disorders include retained fetal membranes (RFM), endometritis, metritis, pyometra, anestrus, repeat breeding and cystic ovarian disease; imposing difficulties for dairy producer/Veterinarian to decide whether to breed, treat or cull such high producing animals as production compromise fertility of the animals due to poor managemental practices and negative energy balance in the transition period [4]. Negative consequences such as delayed uterine involution, prolonged post-partum estrus interval, conception failure and increased services per conception further aid to economic losses [5]. In view of the above constraints in the dairy production systems, an attempt has been made to focus on recent advancements in hormonal therapy, nanotherapy, stem-cell based therapy and gene therapy for more targeted and specific treatment of bovine reproductive disorders and to discuss the possibility of their future applications.

2. Hormonal Therapy

Preparations like Receptal VET (Intervet), Ovulanta (Vet Mankind), Gynarich (INTAS Pharma), Pregulate (Virbac) and Fertygyl (Intervet) as GnRH analogs have been used for the treatment of follicular cysts, anoestrus, delayed ovulation, sub-oestrus and repeat breeding disorder [6]. HCG preparations having LH like activity such as Chorulon (Intervet), Folyson (Indian Immunologicals) are used in the treatment of follicular cysts, anoestrus, delayed ovulation, anovulation, repeat breeding disorder and in males for deficient libido [7]. PMSG preparations having more FSH like activity such as Folligon (Intervet), Trophovet (Indian Immunologicals) and FSH preparations like Folltropin-V (Bioniche, Canada), Super-Ov and FSH-P are used mainly for superovulation in the embryo transfer programme (ETT). Oxytocin preparations like Pitocin (Pfizer) and Syntocinon (Novartis) are used in uterine inertia, RFM and for post-partum uterine involution and milk letdown [8]. $PGF_2\alpha$ (natural) such as Lutalyse (Pfizer), Dinofertin (Alved) as well as synthetic preparations like Vetmate (Vetcare), Clostenol (Zydus AHL), Pregova (Virbac), Repregna (Vet Mankind), Pragma (Intas), Cyclix (Intervet), Oestrushot (Novartis), Estrumate and Illiren (Hoechst, Germany) are used for estrus synchronization in ETT, termination of pregnancy, induction of parturition, fetal mummification, pyometra and luteal cyst [9]. Estrogen preparations like Progynon Depot (Zydus) and Pregheat (Virbac) are used in anoestrus, sub-oestrus, expulsion of retained placenta, fetal mummification and estrus synchronization. Progesterone preparations like Duraprogen (Vetcare), Comshot (Novartis), Hyprogen (Vets Pharma) and proluton Depot (German remedies) are used in threatened abortion, habitual abortion, repeat breeding, post-partum anoestrus and cervico-vaginal prolapse [10]. Progesterone preparations as ear implants such as Crestar (Intervet), Syncro-Mate-B and intra-vaginal implants such as CIDR, PRID and TRIU-B (Virbac) are used in estrus synchronization programme. Intra-vaginal implants are also used in treatment of follicular cysts [11]. Some oral P_4 like MAP, CAP, FGA, Altrenogest are also used for estrus synchronization in other species. Recent research has successfully investigated the use of biodegradable polymers and the potential of delivering several drugs in a continuous or pulsed fashion from a single drug delivery system for the purpose of estrous control of cattle [12]. An electronically controlled intra-vaginal drug delivery system was devised for cattle that achieved multiple drug administration from a single device that used electronics to control the timing (when), rate (how fast) and duration (how long) of drug delivery [13]. The device is capable of delivering a number of drugs (estradiol, prostaglandin and progesterone) at different rates (estradiol and prostaglandin-pulsed delivery; progesterone-continuous release) and times (estradiol 1 h after insertion; prostaglandin 6 days after insertion; progesterone continuously for 10 days). Injectable progesterone encapsulated within biodegradable poly (DL-lactide) microspheres along with estrogen with or without $PGF_2\alpha$ was used to study regulation of estrus and ovulation in mare by Blanchard et al. [14]. One limitation of currently available intravaginal inserts like PRID and CIDR-B is their non-biodegradable nature of silicone core matrix which has to be physically removed in order to terminate treatment. In order to eliminate this activity, it was desirable to develop delivery systems that would not have to be removed from the animal at the end of the treatment period [15]. The development of biocompatible, biodegradable material with non-inflammatory/inert tissue-material interaction fulfilled the desire as platforms for the delivery of progestins. Poly (ϵ-caprolactone) (PCL), a biodegradable polymer incorporated with progesterone and molded in T-shaped intravaginal inserts was studied in ovariectomized cows that mimicked the characteristic plasma profile observed following insertion of a CIDR-B [16]. Recently, it has been shown that the addition of hydroxypropyl β-cyclodextrin (HPβCD) to T-shaped PCL intravaginal inserts contains 10% (w/w) progesterone elevated and sustained plasma progesterone levels nearly 3 times than those seen with the commercially available CIDR-B following intra-vaginal insertion [17]. The addition of polyethylene oxide (PEO) or lactose to the formulation resulted in an insert with more ideal physical properties and cost effective [13]. Hormonal therapy is considered to be effective to induce estrus in delayed pubertal/pubertal anestrus heifers which attain sufficient body weight and show proper genital structures on transrectal palpation. Pubertal heifers having poorly developed genitalia should be kept on a high plane of nutrition along with multivitamins and mineral supplements before initiating hormonal therapies [18].

3. Nanotherapy

Nanomaterials such as metallic nanoparticles, carbon nanotubes, magnetic nanoparticles, quantum dots, fullerenes, liposomes and dendrimers of approximately 1-100 nanometer size are being used for drug delivery, disease diagnosis, treatment, animal nutrition, breeding, and as contraceptives [19]. Nano biosensors are such devices integrated with sensitive probe which are made up of nanomaterials, such as carbon nanotubes, nanowires and nanofibers need validation for the detection of pathogens, oestrus, hormone levels, and metabolites in animals for reproductive management [20]. Nanotube implants based on antibody detection technique are being used to provide real time measurement of changes in estradiol level in the blood as a means of tracking oestrus in animals [21]. Nanoparticle-based vaginal drug delivery has largely been focused on delivering microbicidal drugs in reproductive tract infections enhancing their sustained release, showing low systemic toxicity, allowing targeted treatment to achieve high efficacy [22]. It has been formulated for prevention of sexually transmitted diseases in human as pre-exposure prophylaxis for HIV through maintaining protective drug concentrations between the time of dosing and the time of intercourse [23]. But, the basic physiology of the vagina must be considered while designing nanoparticle based vaginal dosage forms. Nanoparticles based reproductive hormones as nanoemulsions, nanogels, nanocapsules, and liposomes may be used for estrus synchronization and in other reproductive disorders because of their several advantages over other implications. Metal nanoparticles such as cadmium, zinc, nickel and others because of their toxic and deleterious side effects may also be used as contraceptive pills in a dose dependent fashion to target primary reproductive organs for fertility control [24].

4. Stem-cell therapy

In reproductive medicine, stem cells are used for gamete production, endometrial regeneration, amelioration of erectile dysfunction and vaginal reconstruction. Currently, stem cells are considered to be potential therapeutic agents for infertility problems [25].

In vitro production of oocytes from pluripotent cells like embryonic stem cells (ESCs) or induced pluripotent stem cells

(iPSCs) has not proved promising as second-generation primordial germ cells (PGCs) produce fragile and misshapen oocytes, sometimes lacking supporting granulosa cells and even after fertilization of these artificial oocytes, abnormal pronucleus formation has been observed due to faulty epigenetic reprogramming [26]. Formation of functional spermatozoa from iPSCs is reported in mouse but not in human which were capable of fertilizing the oocytes after intracytoplasmatic injection [27]. But, PGC-like cells derived from human iPSCs *in vitro* after transplantation into the mouse testis has been shown to produce functional spermatozoa [28]. There is still need to develop natural testicular niche for a better differentiation of pluripotent cells into functional spermatozoa completely *in vitro*.
Spermatogonial stem cell therapy was shown to be capable of restoring spermatogenesis and fertility upon transplantation in infertile mice. Spermatogonial stem cell transplantation was first time reported in mouse model by Brinster and Zimmermann [29]. Report on donor derived complete spermatogenesis of inter-species spermatogonial stem cell (SSC) transplantation is scarce or negligible in farm animals but, intra-species is reported in almost all farm animals and live progeny has been produced only in sheep and goats [30]. SSC transplantation has been breakthrough for preservation or restoration of male fertility and as an alternative approach for transgenesis and xenotransplantation.
Endometrial regeneration by adult stem cells (ASC) is essential to regain it's function after parturition [31]. It has been shown to repair or regenerate human endometrium *in vitro* and *in vivo* [32, 33]. Bone marrow was found as a source for endometrial ASC [34] and later, bone marrow-derived stem cells (BMDSCs) were successfully used for regeneration of different endometrial cellular compartments. They mainly contributed to the formation of endometrial stromal cells and little to the glandular and luminal epithelium [35, 36]. In animals, destruction and devitalisation of the endometrium which occur in severe metritis, endometritis, pyometra, fetal maceration or in other uterine pathology might lead to obliteration with intrauterine adhesions and loss of functional endometrium in many areas. It cause recurrent pregnancy loss or repeat breeding rendering animal infertile or sterile [37]. Bone marrow-derived stem cells infusion might improve endometrial regeneration if used in affected animals. Although, endometrial stem cells hold great promise for the treatment of infertility related disorders, currently no effective treatment exists for these endometrial pathologies. Yazawa et al. [38] demonstrated that BMDSCs from mouse/human could differentiate into Leydig cells and injection of these stem cells might produce benefits in terms of restoring erectile function and penile physiology. In mouse models, muscle-derived stem cells (MDSCs) seeded on scaffolds were able to regenerate vaginal epithelium by reducing fibrosis and enhancing new tissue formation [39]. So, MDSCs might emerge as potential therapy for vaginal injury during handling of dystocia due to feto-pelvic disproportion and to avoid later pneumo-vagina condition in animals.
There are no stem cell-based therapies available clinically for the treatment of reproductive disorders in case of animals. However, stem cell based therapy can be potential therapeutic approach to a wider array of reproductive problems in animals from endometrial damage to erectile dysfunction, vaginal atrophy and infertility in near future.

5. Gene Therapy

Gene therapy is generally classified in 3 ways. First, *In-vivo* gene therapy means direct administration of gene into organ or animal and *Ex-vivo* gene therapy means *in vitro* transfer of gene in cultured cells from animal and reintroduction of transfected cells in same animal [40]. Secondly, (1) Somatic gene therapy which involve recombinant gene expression in somatic cells for treatment of an inherited or acquired disease for one generation in an individual and (2) Germ-line gene therapy which target only reproductive cells for the elimination of an inherited disease affecting subsequent generations of an individual. Third classification is based on nature or mode of action of introduced gene *i.e.*, corrective gene therapy and cytotoxic gene therapy. Corrective gene therapy is to introduce desired or required gene to correct the genetic or inherited disorders [41]. In contrast, cytotoxic gene therapy aims to destroy abnormal cells by the production of toxic proteins which convert a nontoxic prodrug into a toxic chemical only in abnormal cells or cancerous cells that have high affinity as compared to normal cells [42]. Gene replacement therapy or gene augmentation is suitable for recessive disorders, while, corrective gene therapy is mainly implied for dominant disorders where defective gene has destructive effects in affected animals [43]. Genetic alteration is not only limited for therapeutic purpose like somatic and germ-line genetic alteration in order to treat diseases, but also to non- therapeutic such as genetic enhancement through manipulation at gene level. Most of developmental abnormalities such as gonadal hypoplasia and aplasia, anomalies of the tubular genitalia *i.e.* segmental aplasia of mullerian ducts with persistent hymen (White heifer disease), freemartinism, uterine didelphys, cervical duplex in female and testicular hypoplasia, aplasia or cryptorchidism in male are genetic either autosomal or sex-linked and inherited as dominant or recessive but mostly recessive in nature [44]. These animals are rendered infertile and culled from reproductive stock in farm. Disease conditions mostly encountered at veterinary polyclinic which ultimately affect fertility of animal such as RFM, metritis and cystic ovaries, cervico-vaginal prolapse and mummification are reported to be genetic in nature. Furthermore, high genetic correlation between RFM expressed in first, second and third lactation and between metritis and RFM suggest that the same set of genes may be influencing this disorder irrespective of parity [45]. In other way, we can say genetics play an important role and whole genome sequencing will be fruitful for analysis of defective gene directly or indirectly compromising fertility of animal. Gene therapy based on recombinant DNA technology at preimplantation, fetal or antepartum period in order to culminate genetically determined reproductive disorders will offer great advantages over postpartum treatment. Use of gene therapy in order to eliminate genes regulating placental dysfunction and abnormal uterine contractions in dam will increase chances of survival of fetus till term in pregnancy failure of unknown etiology and will help in selection of competent breeding stock in near future, but currently it's practical implication is far from reach.

6. Conclusion

Potential of delivering several drugs in a continuous or pulsed fashion from a single drug delivery system and use of biodegradable polymers for the purpose of hormonal treatments in animals has led to a more convenient and easy application along with higher potency and minimal side-

effects. Nanobiosensors integrated with nanomaterials such as carbon nanotubes, nanowires and nanofibers need validation for the detection of pathogens, oestrus, hormone levels, and metabolites in animals for reproductive management. Stem cell based therapy can be a potential therapeutic approach to a wider array of reproductive problems in animals from endometrial damage to erectile dysfunction, vaginal atrophy and infertility in near future. Gene therapy can be used to eliminate genes which are harmful and cause reproductive problems in dairy animals. In spite of all these advancements in therapeutic management of bovine reproductive disorders available presently or in near future, nutrition and other aspects of management would remain as major factors regulating homeostasis and diseases in animals especially during transition period. Therefore, most of the reproductive diseases can be prevented if dry cow nutrition and management are well attended during this period.

7. Acknowledgement

The corresponding author would like to thank Director, Indian Veterinary Research Institute for providing financial assistance in the form of institutional Fellowship.

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