Technological Evolution of Hormone Delivery Systems for Estrous Synchronization in Cattle

M.I. Weibel, J.M. Badano and I. Rintoul*

Instituto para el Desarrollo de la Industria Química, Consejo Nacional de Investigaciones Científicas y Técnicas and Universidad Nacional del Litoral, Ruta Nacional 168 Paraje "El Pozo" , CP 3000, Santa Fe, Argentina

*__Corresponding Author:__ irintoul@santafe-conicet.gov.ar

Abstract

This paper reviews the evolution of hormone delivery systems and discusses future technological trends in the field. From intravaginal devices to recent advances in nano and microcapsules will be reviewed. The description of delivery systems manufacture processes, their advantages and disadvantages and the reasons for their success and eventual decline will be described. The increment of the Global demand of animal products, the need for cost reduction, the limited fertile land, the competition with agriculture and the preservation of biodiversity were identified as the driving forces for technological evolution of hormone delivery systems. Micro and nanoencapsulation processes, biomaterials and subcutaneous delivery routes dominate actual research topics. Neuronal targeted drugs administrated through non-invasive routes are speculated as one of the most promising future drug delivery system for estrous synchronization in livestock.

Keywords: Drug Delivery, Animal Reproduction, Artificial Insemination, Ovulation, Microtechnology, Livestock

Introduction

The production of animal products is a key issue for mankind in the XXI century. The way to reproduce animals deals with a number of topics related to technical, biological, economical, social, environmental, regulatory, ethical and health aspects. The analysis of past and actual hormone delivery systems permits to visualize the nature of future technologies. This paper serves as an up-to-date source of information for those involved in farm animal reproduction activities, may facilitate the understanding of driving forces for research and may prepare production units, education programs, regulatory agencies and public opinion to accept new technologies in estrous synchronization.

The demand of animal products is expected to experience a great increase during the XXI century. The offer of animal products could be increased by three basic strategies: increasing the number of animals; increasing the animal productivity through drug enhanced metabolism and increasing the animal productivity through genetic improvement. The first option is not long

term sustainable. The limited amount of fertile land, the competition with agricultural and urban spaces and the everyday more restricted access to new land in detriment of biodiversity and natural forests can be mentioned as the main reasons for such statement. The second option is under discussion. On the one hand, the United States policy is rather permissive in the use of drugs such as anabolic steroids and growth promoting hormones for the acceleration of the animal metabolism. On the other hand, the European policy is quite restrictive in altering the animal metabolism. The policy of Latin American countries tends to follow the European normative since the European Community is the main exportation market of the Latin American livestock production. Finally, the third option seems to be reasonable without restrictions except those implying ethical issues related to gene modification through gene engineering techniques. Genetic breed improvement through selective crossbreed has been used by mankind during centuries. The development of artificial insemination (AI) techniques accelerated the breed improvement process while keeping its essence as an ethically right procedure.

Globally, near 20% of the bovine breeders, 110.5 million animals per year are artificially inseminated. Far Orient and Oceania, Europe, North America and South America register 58.2, 37.7, 11.2 and 1.4 million operations annually, respectively (Thibier and Wagner, 2002).

The artificial induction and synchronization of estrus in production animals is critical to ensure a positive balance of the cost-benefit equation of the AI related activities. The costs related to the selection and extraction of quality semen, the conservation and logistics of semen, the detection of estrus and the insemination of animals are compensated only if a large number of animals in estrous are simultaneously treated.

Hormone treatment is the usual method to induce estrus and ovulation in production animals. The administration of hormones must be very precise. The bioavailability of the hormones should be keep within strict levels to induce estrus and ovulation without negative side effects.

The controlled hormone release is a current technological challenge. A multitude of disciplines such as materials science, animal physiology, production processes, application techniques and farm supply logistics among others converge in the development of hormone delivery systems (HDS). Several HDS have been developed for the controlled release of one or more hormones according to defined profiles. These HDS have evolved during more than 40 years according to the technological advance of a multitude of disciplines, sometimes not directly related to reproductive veterinary. Nowadays, HDS represent about 15% of the global market of veterinary

products (Evans, 2010).

This paper reviews the technological evolution of HDS for the artificial induction and synchronization of estrus and ovulation in cattle. Moreover, a brief explanation of the driving forces for the development of the time ago called "new" concepts and associated technologies and the reasons of their decline, obsolescence and replacement by other technology is included. Finally, the future technological trends based on the recent advances on biomaterials and nanotechnology will be discussed.

The problem of animal reproduction

Globally more than 90% of the animal reproduction is carried out without human intervention (Barbosa and Machado, 2008). The reason for such situation is the difficulty to control the estrus cycle and to detect ovulation in the animals as preliminary condition for AI. Normally, the fertile period of bovine breeders ranges between 24 to 48 h. Besides, animals from the same herd ovulate several weeks apart (Ball and Peters, 2004). The very short fertile period and the high temporal dispersion of ovulation within the herds implies that the staff responsible for carrying out the insemination should be present almost every day at the field to run a detailed examination of each animal, identify those in the fertile period and eventually inseminate them. In addition, the total cost of the operation is increased by the consequences of the poor accessibility to the herds due to the large distances between urban centers and livestock locations, the precarious state of rural roads, the strict requirements of the logistics of semen and the complication of activities carried out in open field conditions. Finally, the animal reproduction assisted by AI became unprofitable for the common livestock producer.

The ultimate consequences of this situation are a significant drawback in the increase of the genetic charge of the herds and associated loss of productivity. Moreover, the profitability of the livestock space diminishes as a consequence of large periods for deliveries, awkwardness for the assistance and control of calves of different ages, loss of efficiency of vaccination and feeding programs and the low performance of the involved staff.

The main advantages that would bring the synchronization of estrus in livestock are: a substantial increase in the rate of genetic breed and herds improvement, the concentration of animals in estrus in a short period, the rationalization of AI, the concentration and reduction of the calving period, the streamlining of vaccination programs, the improvement of feeding programs according to the season and the age and breed of the animals, a greater ease in implementing

Page 22

zootechnical tests to determine trading parameters, simpler traceability of animals and better management and logistics of animals (Becaluba, 2006).

Developed technologies and drug delivery routes

Several technologies have been developed with the aim of controlling the estrus cycle in cattle. They all imply the administration of drugs and hormones with different functions in the reproductive metabolism of the animals. Several polymeric matrices have been used for the sustained release of drugs and hormones in order to obtain well determined drug concentration and profiles during a specific time. Matrices have been designed in various shapes and can be placed in different sites of the animal to activate its function. According to the application site, the matrices can be classified in four categories: intravaginal delivery systems, subcutaneous delivery systems, respiratory route delivery systems and dermal delivery systems.

Intravaginal delivery systems

Intravaginal devices were developed in the 1970's by taking advantage of the possibilities offered by extrusion, injection molding and lamination of thermoplastics. They are the most currently applied HDS. A brief description of the devices and their manufacturing processes is presented below.

The Progesterone Releasing Intravaginal Device (PRID) comprises 1-3 g of micronized progesterone, uniformly suspended in a silicone rubber matrix which is cured onto a stainless steel spiral. A progesterone mixture suspended in liquid silicone rubber is injection molded around a 3.5x28.5x0.1 cm3 stainless steel plate. Then, the silicone matrix is cured inside the mold, so that it becomes an elastic semisolid. It is removed from the mold and formed into a coil with a diameter of approximately 4 cm and a length of approximately 12 cm (Rathbone et al, 1997). Figure 1 shows a scheme of a PRID. The release rate of progesterone depends almost exclusively on the contact area between the device and the vaginal epithelium of the host animal. The retention rate of these devices is usually very high, usually over 95%. Although, it has been initially designed for cows, with minor modifications it can be also used in mares (Rathbone et al, 1997).
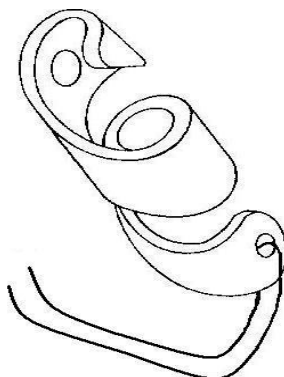
Figure 1

Controlled Internal Drug Release (CIDR) devices are made of a "T" or "Y" shaped nylon spine manufactured by injection on which it is deposited, by automated injection molding, a layer of 0.9-5.0 mm of silicone rubber impregnated with 1.9 g of micronized progesterone (Macmillan and Peterson, 1993). Figure 2 shows the scheme of a CIDR. The device has two wings used to exert a small pressure in the walls of the vagina in order to ensure its retention. Usually, retention rates in cows are over 95%, but they are lower in case of mares and buffalos. The great technological improvement of the CIDR is the replacement of the metallic soul and the spring shape of the PRID by a cheaper and more flexible nylon soul and a more anatomic Y shape, respectively.
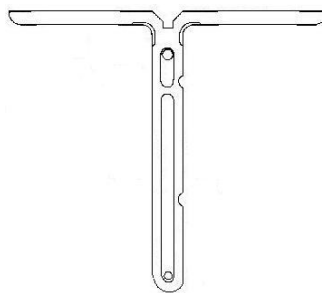
Figure 2

Numerous sponge devices have been developed and studied academically. However, few of them have been produced in mass and converted in commercially available products. Its application is limited almost exclusively to sheep. The sponges present different lengths, diameters, densities, porosities and consistence. Generally, they are produced by processes similar to those used to obtain polyurethane foam. In fact, polyurethane foam is the most extended material used for the manufacturing of sponge based HDS. Those processes comprise the dissolution of the polymer in a low boiling point liquid solvent. Then, the solvent is rapidly volatilized obtaining a sponge of

Page24

the polymer previously dissolved. The intravaginal device is obtained through the pulverization of a hormone solution in a volatile solvent, such as alcohol (Carrick and Shelton, 1967) or acetone (Davis et al, 1983), on the surface of the sponge. This method has been used to obtain sponges impregnated with progesterone (Scanlon et al, 1971) and estradiol (Davis et al, 1983), among others hormones with proven activity in the regulation of the reproductive cycle. Once the sponges have been impregnated, the solvent evaporates and the hormones remain finely deposited on the large surface of the sponges (Hale and Symington, 1969). An alternative procedure is the immersion of the base sponge in a bath of hormone solution. Then, the sponge is removed from the bath and the solvent is vaporized, obtaining a hormone dispersion over the sponge surface (Shimizu et al, 1967). It is usual the addition of antibiotics to these devices because its greater specific surface increase the possibility of microbial colonization. Figure 3 shows a basic scheme of this sponge-like intravaginal device.
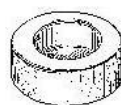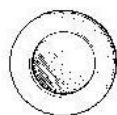
Figure 3

The Intravaginal Application System (INVAS) comprises a "T"-shaped flexible polypropylene structure with a length of approximately 14 cm, covered with a progesterone-impregnated silicone rubber skin. Its shape is similar to the CIDR. The progesterone addition to the silicone matrix is done through a lamination and milling process, similar to that used in the incorporation of sulfur and black carbon to the rubber in tire production. In this case, the material is treated as a paste. This paste is laminated and cut in a T-shape. A silicone-polypropylene-silicone sandwich is obtained inside a mold. The mold is closed and heated to cure the silicone and seal the sandwich structure (Hornykiewytsch, 1988). This method allows the use of plastics of lower melting point than in the CIDR case and the progesterone does not change its crystalline structure during the process (Hiller and Hornykiewytsch, 1995).

The Ring-like Intravaginal Device was extensively investigated but with no commercial success. It comprises a plastic or steel ring covered with a hormone-impregnated silicone rubber skin. The major disadvantage of this device is its poor retention in the vagina, which in no case has exceeded 3 days (Roche, 1976). Figure 4 shows the diagram of a ring-like intravaginal device.
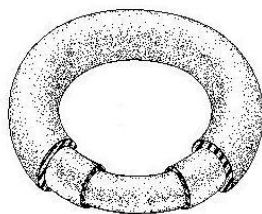

Figure 4

The Rajamahendran intravaginal device is obtained from two silicone tubes with a length of approximately 20 cm, an inside diameter of 0.8 cm and an outside diameter of 1.3 cm. A diethyl ether solution of progesterone is poured in the tubes, then the solvent is vaporized and the tubes are sealed. An estradiol paste is dispersed in a layer around the ends of the silicone tubes. Finally, both tubes are centrally tied together forming a cross. Strings can be fastened to the tubes to facilitate the removal from the vagina (Rajamahendran et al, 1982). Figure 5 shows a scheme of this device.
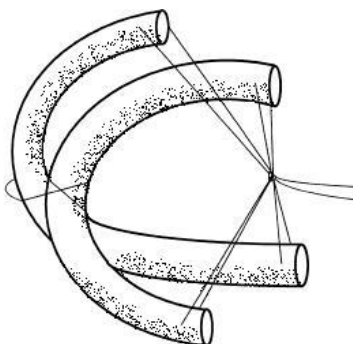

Figure 5

The Intelligent Breeding Device (IBD) allows the release of different hormones such as progesterone, prostaglandin, and estradiol with rates perfectly defined at specific times. This

device comprises a head connected to a container. The head has tubular flexible arms that facilitate the introduction of the device, its retention in the vagina and the hormone release. The container is a hermetically-closed, rigid plastic tube with a length of approximately 12 cm and a diameter of 4 cm. The controlling chip, the plunger micropumps, the hormone reservoirs and the batteries are arranged inside the container. The chip and the micropumps are programmed in order to control the rates and times of release of the different hormones (Jellie, 2002). Figure 6 shows the external view of an IBD. This device was discontinued due to its complexity and disappointing field trials.
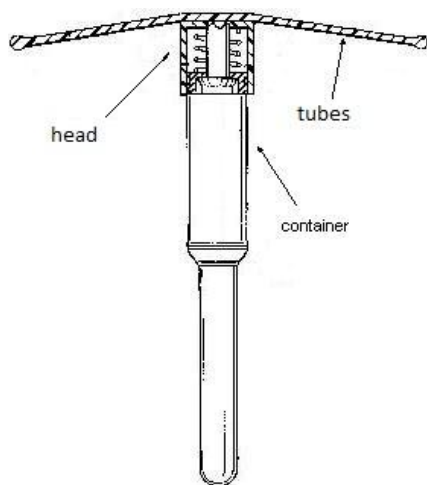

Figure 6

All the intravaginal devices intend to release in a sustained way, one or various hormones with the aim of control the estrus cycle of the animal. These devices must be introduced in the animal vagina to activate their function. Their big size and geometrical complexity make difficult its production, storage and transport. Their use usually involves the following steps:

1) Immobilize the animal so as to have access to the rear. This task is not always easy to accomplish on the precariousness of the country.

2) Clean up the vaginal area of the animal to minimize the risk of infection. It is essential to use gloves, cleaning agents, disinfectants and especially large amount of clean water under certain pressure. It is important to mention that these requirements are many times difficult to accomplish in the precarious conditions of the country.

3) Carefully insert the device into the vaginal cavity of the animal, avoiding any contact that could lead to transfer some hormone from the device surface to the skin of the operator. It implies the mandatory use of gloves. The gloves must be replaced from animal to animal to avoid the eventual propagation of venereal diseases through the herd. The use of gloves has important economic consequences due to the costs of related logistics and final disposal actions. The insertion of intravaginal devices should be performed by trained and skilled personal, which means an extra cost in education and training. The serial production of intravaginal devices makes virtually impossible to manufacture customized devices. Therefore, the devices are fabricated with a dosage, size and shape according to the average animal. Thus, there is always a risk of under or over dosage because the optimal dose may vary from animal to animal. In addition, the vaginal anatomy varies greatly in size, physical consistency and specific particularities, depending on the animal size, breed, age, previous deliveries, etc. Consequently, the device may not enter or come loose falling out the vagina.

4) The device must be removed from the vagina after a 7-10 days period. The animal must be immobilized and hygienized again. The device should be taken out by qualified personnel, with appropriate security conditions and the mandatory use of gloves. Commonly, the exhausted devices may present a hormone residue of 40-60 wt% and the propagation of venereal diseases is always present.

5) Finally, the exhausted devices and used gloves must be compulsory burned or buried as final disposition act to prevent any future accidental contact with the human skin.

It is common that the use of intravaginal devices is accompanied with the intramuscular injection of supplementary hormones. The following describes a protocol for the use of a commercially available intravaginal device. Day 0: immobilize the animal, clean the vaginal area, insert the intravaginal device, and inject 2 mg of estradiol benzoate intramuscularly. Day 8: immobilize the animal, clean the vaginal area, remove the device, and inject a dose of prostaglandin. Day 9: inject estradiol benzoate; 1 mg in cows and 0.75 mg in heifers. Day 10: detect heat and inseminate or use the fixed time AI (Pfizer Sanidad Animal, 2012).

From the beginning of the treatment to the insemination day, each animal was handled 10 times. Thus, the final cost of the operation results quite high and the logistics and coordination of specialized personnel involved is very complex.

Herein lays the reasons for the developing of subcutaneous delivery systems.

Subcutaneous delivery systems

Two big fields can be distinguished in this route of hormone administration: implantable and injectable systems. The implantable systems generally require a surgery to access the subcutaneous tissues and implant there the delivery system. The injectable systems access these tissues through the use of veterinary needles.

The HDS implantable under the skin was the first subcutaneous delivery system to be developed. These systems comprise tubes (Short et al, 1976) or disks (Scanlon et al, 1971) made of silicone or other biocompatible polymers, not necessarily biodegradable, that have been loaded in some way with progesterone or another hormone. These systems are implanted under the skin of the animal with a small surgery in order to allow the release of the hormone directly to the animal's body. In these cases the quality and the purity of the hormone and the size and the shape of the implant have crucial importance. The effectiveness of these delivery systems is generally pretty good. However, the dosage problem remains because the implants are manufactured with a unique hormonal load. In some cases, additional surgery is necessary for the removal of exhausted systems. The large size of the implants, the need to immobilize the animal for the surgery, the need for proper aseptic conditions in the surgery, and eventually the double sequence of tasks related to the removal of the exhausted implant lead to serious doubts about the feasibility of implementing these types of HDS.

The subcutaneous delivery systems implanted in the ear were designed as a way to facilitate the operations of implanting and removal of the devices. Several types of ear implants and its implanting devices have been developed at commercial scale. These implants are polyurethane or silicone tubes with at length of 20 mm and a diameter of 3 mm. They contain between 5-15 mg of hormones homogeneously distributed in the polymer matrix (Chien and Lau, 1976). The implants are stored with protective sheaths. They are put in the implanting device and introduced

into the animal's ear with relative ease. The technique greatly simplifies the surgery work. The removal of the implant is made with a small incision. However, as in previous cases, the hormone residue is between 50 and 65 % of the initial load (Moseley et al, 1979), so the dosage problem is still remaining and it has also been proved that the hormone release rate is not constant during the treatment (Kesler et al, 1995). The Microsealed Drug Delivery (MDD) technology was developed as an attempt to keep constant release rate. These types of HDS are similar to the previous one being the only difference that the hormone is contained in polyethylene glycol reservoirs dispersed in the polymer matrix (Chien, 1982). Figure 7 shows a scheme of the application of an ear-implanted delivery system.
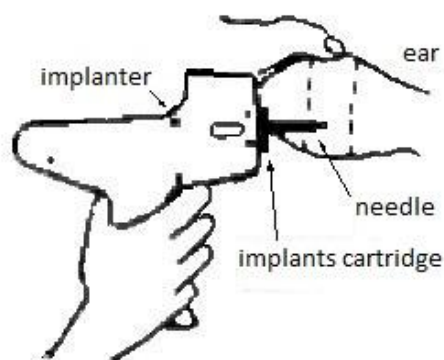

Figure 7

The injectable systems appeared in the last decade of the XX century, as an alternative to eliminate the surgery needed to implant and remove the implantable delivery systems and the dosage problem. An injection does not need rigorous aseptic conditions; it is simpler and quicker that an implant and it has the possibility of varying the doses by adjusting the injected volume, without the need for highly qualified personnel. Once injected, it is not possible to remove the delivery system, so it is necessary to design it with biocompatible and biodegradable matrices capable to release the hormones with a zero order pattern during a period of 7-10 days followed by a sudden decay. The injectable delivery systems are classified in three categories: transdepot, preformed systems and in situ formed systems.

The transdepot systems comprise liquid hormonal solutions, with variable viscosity, where the hormone is dispersed or in solution. The system is always liquid when injected. Once injected, the system becomes a semisolid by some physical-chemical change induced by the environment of the injection site. This technology was initially developed for human medicine (Tsung and

Page30

Burgess, 2012). Then, it evolved to veterinary medicine. In most cases, the simple transfer of an application in humans to animals results with some technical success but it is highly unfeasible from an economic point of view. The so-called thermoplastic pastes are injected as a molten liquid at a temperature higher than those of the injection site. Once injected, the liquid is cooled to the animal's body temperature, forming a semisolid depot and entrapping the hormonal load (Bezwada, 1995). The hormonal load is released to the environment according to the structure and characteristics of the depot. The most used materials for the manufacturing of these systems are biodegradable, biocompatible, low molecular weight polymers and copolymers derived from the lactic acid, glycolic acid, caprolactone, trimethylene carbonate, dioxanone, and ortho esters (Bezwada et al, 1997; Einmahl et al, 2001). However, it is important to note that the melting points of these polymers are above 60°C, so its injection could be very painful and produce necrosis and crusts in the adjacent zone (Liu and Wilson, 1998). On the other hand, various geometric shapes could be adopted by the depots during their injection, from beads to tree or spider shapes. Different shapes result in a wide dispersion of the release rates due to the random and uncontrollable variation of the contact area. Relatively low release rates are also observed in these systems (Dordunoo et al, 1997). Another transdepot injectable system is the in situ cross-linking type. A liquid solution is prepared containing monomers, polymers, the cross-linking agent and the drugs, which could be dispersed or dissolved. This solution is injected into the animal. Once injected, the cross-linking agent is activated by some physical-chemical stimuli, polymerizing and linking polymeric chains, forming a semisolid with the drug load trapped inside (Dunn et al, 1994). The initiators must be added to the solution some seconds before the injection to avoid cross-linking in the syringe. The toxicity of the monomers and cross-linking agents, the ability to inhibit the reaction of physiological conditions and body fluids, and the temperature rise due to highly exothermic reaction of polymerization and curing that may seriously affect surrounding tissues can be mentioned as limiting facts in the application of such systems (Peter et al, 1997; Zhao et al, 2000). The most recent transdepot systems are the so-called ion-induced gelation systems. They comprise biopolymers that are capable of gelling in the presence of polyvalent cations. Alginate and albumin in combination with calcium are the most studied (Viegas et al, 1994; Cui and Messersmith, 1998). Finally, it must be mentioned the injectable delivery systems formed by polymer precipitation. In these cases, a mixture is prepared containing a biodegradable and biocompatible polymer, generally derived from lactic

Page31

acid, glycolic acid and caprolactone, a biocompatible and bioassimilable solvent, such as pyrrolidone and dimethyl sulfoxide, and the hormone load. This mixture must be homogeneous and stable while stored. Once injected, the precipitation of the polymer is induced in the injection site, forming a solid or semisolid structure with the drug trapped inside (Dunn et al, 1990). The hormone is released to the environment according to the characteristics of the formed structure. Generally, the polymer precipitates by solvent removal (Shah et al, 1993), temperature changes (Jeong et al, 2000) or pH changes (Siegel et al, 1988). The addition of surfactants allows controlling the polymer precipitation in form of massive elements, sponges, particles and microparticles. The limitation of such procedure is the high level of drug release just before and in the early stages of the polymer precipitation (Shively et al, 1995; B.L. Chandrashekar et al, 2000). This causes serious irritations in the adjacent tissues and can even be toxic to the host organism. Besides, most of the solvents and eventually surfactants used in the formulation of the depots are toxic, potentially hemolytic, with necrotic power and can damage muscle activity (G. Chandrashekar and Udupa, 1996).

The situation previously discussed and the recent development of micro- and nanotechnologies are the driving forces for the development of delivery systems comprising microparticles. Microparticle is a general term used to identify microspheres, microbeads and microcapsules. In the context of veterinarian products, the word micro usually refers to systems able to flow and be injected through veterinary needles. The method consists in preparing microparticles, generally microspheres. The hormonal load is trapped or encapsulated during such process. These microparticles are dispersed in a carrier liquid and injected into the animal. There are no less than 18 techniques to obtain microparticles, of which 11 would be appropriate to obtain preformed, hormone loaded microparticles. Each technique has particular advantages and disadvantages. The main challenge is to select the most appropriated technique for a given set of load, material and application. Following, it is presented a brief description of each technique.

The emulsion method consists in the dispersion of two immiscible phases, being the drug soluble just in the discontinuous phase. Subsequently, the continuous phase is removed by spray drying leaving the drug encapsulated in the dispersed phase (Frangione-Beebe et al, 2001; Benita and Tamilvanan, 2006).

Page 32

The internal gelation technique comprises the dissolution of the drug and a polysaccharide in water. This solution is dispersed in an immiscible oil phase containing a polysaccharide gelling precursor dissolved. The addition of a destabilizing agent, generally Ca2+, induces the gelation of the dispersed phase, trapping the drug in this matrix (Poncelet, 2001).

The encapsulation method by phase separation forms a drug suspension using a hydrophilic polymer. Then, the polymer precipitation is inducted and the drug remains trapped inside the precipitated microparticles (Bachtsi et al, 1996; Lamprecht et al, 2000).

The interfacial polymerization method consists in dispersing or dissolving the drug load in a monomer solution, which is then dispersed in an oil phase containing the polymerization initiator. The polymerization occurs in the oil interface, leaving the content encapsulated inside the monomeric phase (Levy and Andry, 1991; Kulkarni et al, 2000).

The atomization method consists in dispersing a polymer/drug solution in a gas phase with sudden evaporation of the solvent. This process leaves the drug trapped in a polymer matrix (Abraham et al, 1996).

The desolvation technique comprises the dissolution of a drug and a wall-forming material in a small quantity of solvent. This solution is then extruded or dispersed in a medium with excess of non-solvent liquids. The non-solvent desolvates the solution leaving the content trapped in a matrix constituted by the wall-forming material (Lamprecht et al, 2000).

The centrifugal extrusion method consists in pumping a solution of the wall-forming material and a solution or dispersion of the drug through a dual-head rotor. The drug solution passes through the internal head and the wall-forming material solution passes through the external head. The centrifugal force breaks the stream forming drops of drug covered by the wall-forming material. These drops become capsules upon contact with a solution of the gelling agent of the wall-forming material (Gibbs et al, 1999).

The rotating disk atomization method consists in dispersing a drug/wall-forming material solution in a liquid film over a rotating disk. When the fluid reaches the edge of the disk is ejected as droplets. These droplets become capsules upon contact with a hardening solution (Ogbonna et al, 1989); Senuma et al, 2000).

In the jet cutting method the drug/wall-forming material solution is passed through a hole forming a continuous stream which is cut by a rotor with multiple blades. The drops will then fall

Page33

into a gellant solution to form the capsules (Prüße et al, 1998).

Finally, in the encapsulation by electrostatic dripping or by vibrating extrusion, the drug/wall-forming material solution passes through needles. Each forming droplet in the tip of the needle is pulled from it by means of an electrostatic force or by mechanical vibration as appropriate (Hulst et al, 1985; Bugarski et al, 1994; Hallé et al, 1994).

Two or more of these techniques could be combined to produce multilayer microcapsules. In general, the most appropriated techniques for producing large volumes such as emulsion or atomization encapsulation, give very small particles with wide dispersion of sizes and properties. By contrast, techniques that allows precise control of size, size distribution and particle properties, have much lower production volumes and are not ease to scale-up.

Delivery systems by respiratory route

A recent way of induction of estrus in programmed reproduction of animals is the controlled pheromone delivery by respiratory route. The technique is very recent and still not fully developed. It comprises the microencapsulation by atomization of very small doses of pheromones that are then administered to animals by inhalation using a single dose. The activation of the animal's hormonal metabolism is the great advantage of this technique. Pheromones have the ability to wake up the hormone production system through very specific actions in hypothalamic centers (Whitten, 1999; Rekwot et al, 2001; Villeneuve, 2001). Another developing technique is the use of free or encapsulated progesterone for nasal administration. In the first case, progesterone microcrystals, obtained by antisolvent or combined antisolvent and cooling crystallization (Ragab et al, 2010), or progesterone suspensions in isotonic solutions of polyethyleneglycol are used (Corbo et al, 1988), while in the second case ciclodextrins are the most employed materials for the encapsulation of progesterone in order to enhance the solubility and absorption of the steroid (Van den Berg et al, 2004; Rathnam et al, 2008). The potential of this technology is to avoid the first-pass metabolism, increasing therefore the hormone half-life. However, the use of nasal spray devices is expensive and difficult to apply to large herds in open field.

Delivery systems by dermal route

The skin is one of the most important organs of the body. It is a complex membrane, comprising three layers: the dermis, the epidermis and the outermost layer, the stratum corneum. The latter

Page34

acts as a barrier towards the penetration of different objects and molecules, and controls the permeation and release of active compounds. Several veterinary drugs are being formulated into topical products. Various approaches have been studied to develop dermal formulations. One approach includes the development of emulsions and microemulsions containing a continuous aqueous phase, a dispersed oil phase with the lipophilic hormone and surfactants. These formulations usually includes skin permeation enhancers, such as surfactants, cyclodextrins, fatty acids, chitosan derivatives and chelators. The second approach consists in the development of membrane reservoirs hydrogels containing the hormone. In this case, different polymers have been used to obtain a zero order release, such as chitosan derivatives, polyacrylic acid derivatives, cellulose derivatives, etc. Transdermal patches are divided in matrix type, which contain an additional adhesive layer or the matrix is self-adhesive, and membrane controlling type consisting in a rate controlling membrane of poly(ethylenvinylacetate) or polyehtylene. In these cases the drug is dissolved or suspended in a lipophilic membrane (Valenta and Auner, 2004). Recently, liposomes (Biruss and Valenta, 2006) and polymer nanoparticles (Tomoda et al, 2012) have been investigated for the transdermal delivery of steroids. These systems overcome the first-pass metabolism and can deliver a constant dose of drugs for long time (Prausnitz and Langer, 2008). Despite the fact that the skin has the same three layers in all the animals of interest, there are important specie-specie and specie-climate variations in epidermal anatomy and physiology that discourage the extended use of a single transdermal delivery system (Monteiro-Riviere, 1990). Besides, the use of permeation enhancers leads to potential skin irritation (Jordan et al, 1998).

Conclusion and future perspective

The increasing demand for animal products is the main driving force for the improvement of animal reproduction programs and techniques. The steady decline in grazing land, either by desertification, competition with agriculture, urban and biodiversity reserves, the restrictions from health and food safety agencies and the ethical issues involving artificial gene modification of animal food products lead the development of natural genetic improvement of farm species by cross-breeding programs which lead directly to the development of drug delivery systems for control and synchronization of estrus and ovulation cycle, as previous steps for AI. The trend is to develop delivery systems more economic, with simple and robust production processes, less

invasive, with no long-term effects in animals, ease to apply and dose and secure for those who handle it. It has been observed a gap of 30 to 40 years from conception of delivery systems to commercially availability. It has also been seen several developments that has never come to production and commercialization stages. The explosion of micro- and nanotechnologies currently proposed a wide spectrum of techniques. Over time the less efficient will be discarded and those with higher yields and lower cost/benefit ratio will be refined. Despite the great efforts in the development of more efficient HDS the market is dominated by CIDR type intravaginal devices. However, more than 90% of the global animal reproduction business is still waiting for a technically efficient and economically profitably new technology for controlled hormone delivery.

References

1. Abraham SM, Vieth RF and Burgess DJ. 1996. Novel technology for the preparation of sterile alginate-poly-l-lysine microcapsules in a bioreactor. Pharmaceutical Development and Technology. 1: 63-68.
2. Bachtsi AR, Boutris CJ and Kiparissides C. 1996. Production of oil-containing crosslinked poly(vinyl alcohol) microcapsules by phase separation: effect of process parameters on the capsule size distribution. Journal of Applied Polymer Science. 60: 9-20.
3. Ball PJH and Petters AR. 2004. Reproduction in Cattle, 3rd edition. Blackwell Publishing Ltd, Oxford, UK.
4. Barbosa RT and Machado R. 2008. Panorama da inseminação artificial em bovinos. Embrapa Pecuária Sudeste, São Carlos, Brazil.
5. Becaluba F. 2006. Métodos de sincronización de celos en bovinos. Retrieved 30 July 2012,from
http://www.produccionbovina.com/informacion_tecnica/inseminacion_artificial/92-metodos_sincronizacion.pdf
6. Benita S and Tamilvanan S. 2006. Lipid and polymeric colloidal carriers for ocular drug delivery. In Microencapsulation: methods and industrial applications (ed. S Benita), pp. 587-624. CRC Press, Boca Raton, FL.
7. Bezwada RS. 1995. US Patent No. 5442033.
8. Bezwada RS, Arnold SC, Shalaby SW and Williams BL. 1997. US Patent No. 5653992.
9. Biruss B and Valenta C. 2006. Skin permeation of different steroid hormones from polymeric coated liposomal formulations. European Journal of Pharmaceutics and Biopharmaceutics. 62: 210-219.
10. Bugarski B, Li Q, Goosen MFA, Poncelet D, Neufeld R and Vunjak G. 1994. Electrostatic droplet generation: mechanism of polymer droplet formation. AIChE Journal. 40: 1026-1031.
11. Carrick MJ and Shelton JN. 1967. The synchronization of oestrus in cattle with progestagen-impregnated sponges. Journal of Reproduction and Fertility. 14: 21-32.
12. Chandrashekar BL, Zhou M, Jarr EM and Dunn RL. 2000. US Patent No.6143314.

Page36

13. Chandrashekar G and Udupa N. 1996. Biodegradable injectable implant systems for long term drug delivery using poly (lactic-co-glycolic) acid copolymers. Journal of Pharmacy and Pharmacology. 48: 669-674.

14. Chien YW. 1982. Controlled administration of estrus-synchronizing agents in livestock. In Novel drug delivery systems: fundamentals, developmental concepts, biomedical assessments (ed. YW Chien), pp. 413-463. Marcel Dekker, New York.

15. Chien YW and Lau EPK. 1976. Controlled drug release from polymeric delivery devices IV: in vitro-in vivo correlation of subcutaneous release of norgestomet from hydrophilic implants. Journal of Pharmaceutical Sciences. 65: 488-492.

16. Corbo DC, Huang YC and Chien YW. 1988. Nasal delivery of progestational steroids in ovariectomized rabbits . I . Progesterone - comparison of pharmacokinetics with intravenous and oral administration. International Journal of Pharmaceutics. 46: 133-140.

17. Cui H and Messersmith PB. 1998. Thermally triggered gelation of alginate for controlled release. In Tailored polymeric materials for controlled delivery systems (eds. I McCulloch and SW Shalaby), pp. 203-211. ACS Publications, Washington, DC.

18. Davis SR, Welch RA, Pearce MG and Peterson AJ. 1983. Induction of lactation in nonpregnant cows by estradiol-17 beta and progesterone from an intravaginal sponge. Journal of Dairy Science. 66: 450-457.

19. Dordunoo SK, Oktaba EMC, Hunter W, Min W, Cruz T and Burt HM. 1997. Release of taxol from poly(ϵ-caprolactone) pastes: effect of water-soluble additives. Journal of Controlled Release. 44: 87-94.

20. Dunn RL, English JP, Cowsar DR and Vanderbilt DP. 1990. US Patent No. 4938763.

21. Dunn RL, English JP, Cowsar DR and Vanderbilt DP. 1994. US Patent No. 5278201.

22. Einmahl S, Capancioni S, Schwach-Abdellaoui K, Moeller M, Behar-Cohen F and Gurny R. 2001. Therapeutic applications of viscous and injectable poly(ortho esters). Advanced Drug Delivery Reviews. 53: 45-73.

23. Evans T. 2010. Beef Outlook. Vetnosis Ltd, Edimburgh, UK.

24. Frangione-Beebe M, Rose RT, Kaumaya PTP and Schewendeman SP. 2001. Microencapsulation of a synthetic peptide epitope for HTLV-1 in biodegradable poly(D,L-lactide-co-glycolide) microspheres using a novel encapsulation technique. Journal of Microencapsulation. 18: 663-677.

25. Gibbs BF, Kermasha S, Alli I and Mulligan CN. 1999. Encapsulation in the food industry: a review. International Journal of Food Sciences and Nutrition. 50: 213-224.

26. Hale DH and Symington RB. 1969. Control of sexual activity in ranch cows by intramuscular and intravaginal administration of progestagens. Journal of Reproduction and Fertility. 18: 193-199.

27. Hallé JP, Leblond FA, Pariseau JF, Jutras P, Brabant MJ and Lepage Y. 1994. Studies on small (< 300 microns) microcapsules: II-Parameters governing the production of alginate beads by high voltage electrostatic pulses. Cell Transplantation. 3: 365-372.

28. Hiller D and Hornykiewytsch T. 1995. US Patent No. 5398698.

29. Hornykiewytsch T. 1988. Intra-vaginal application system (INVAS) for controlled drug release in animals. Acta Pharmaceutica Technologica. 34: 68-79.

30. Hulst AC, Tramper J, Van't Riet K and Westerbeek JMM. 1985. A new technique for the production of immobilized biocatalyst in large quantities. Biotechnology and Bioengineering. 27: 870-876.

31. Jellie HP. 2002. US Patent No. 6375649 B1.

32. Jeong B, Bae YH and Kim SW. 2000. In situ gelation of PEG-PLGA-PEG triblock copolymer aqueous solutions and degradation thereof. Journal of Biomedical Materials Research. 50: 171-177.

33. Jordan WP, Atkinson LE and Lai C. 1998. Comparison of the skin irritation potential of two testosterone transdermal systems: an investigational system and a marketed product. Clinical Therapeutics. 20: 80-87.

34. Kesler DJ, Favero RJ and Troxel TR. 1995. A comparison of hydron and silicone implants in the bovine norgestomet and estradiol valerate estrus synchronization procedure. Drug Development and Industrial Pharmacy. 21: 475-485.

35. Kulkarni AR, Soppimath KS, Aminabhavi TM, Dave AM and Mehta MH. 2000. Glutaraldehyde crosslinked sodium alginate beads containing liquid pesticide for soil application. Journal of Controlled Release. 63: 97-105.

36. Lamprecht A, Schäfer UF and Lehr CM. 2000. Characterization of microcapsules by confocal laser scanning microscopy: structure, capsule wall composition and encapsulation rate. European Journal of Pharmaceutics and Biopharmaceutics. 49: 1-9.

37. Levy MC and Andry MC. 1991. Mixed-wall microcapsules made of cross-linked proteins and polysaccharides: preparation and properties. Journal of Microencapsulation. 8: 335-347.

38. Liu SY and Wilson BC. 1998. Hyperthermia and photodynamic therapy. In Basic science of oncology (eds. I Tannock and RP Hill), pp. 443-453. McGraw-Hill, New York.

39. Macmillan KL and Peterson AJ. 1993. A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrous synchronisation, increasing pregnancy rates and the treatment of post-partum anoestrus. Animal Reproduction Science. 33: 1-25.

40. Monteiro-Riviere NA. 1990. Comparative anatomy, physiology, and biochemistry of mammalian skin. In Fundamentals and methods of dermal and ocular toxicology (ed. DW Hobson), pp. 1-23. CRC Press, Boca Raton, FL.

41. Moseley WM, Forrest DW, Kaltenbach CC and Dunn TG. 1979. Effect of norgestomet on peripheral levels of progesterone and estradiol-17β in beef cows. Theriogenology. 11: 331-341.

42. Nash HA and Mishell Jr. DR. 1981. US Patent No. 4292965.

43. Ogbonna JC, Matsumura M, Yamagata T, Sakuma H and Kataoka H. 1989. Production of micro-gel beads by a rotating disk atomizer. Journal of Fermentation and Bioengineering. 68: 40-48.

44. Peter SJ, Nolley JA, Widmer MS, Merwin JE, Yaszemski MJ, Yasko AW, Engel PS and Mikos AG. 1997. In vitro degradation of a poly(propylene fumarate)/β-tricalcium phosphate composite orthopaedic scaffold. Tissue Engineering. 3: 207-215.

45. Pfizer Sanidad Animal 2012. Protocolo de uso del CIDR. Retrieved 30 July 2012, from https://animalhealth.pfizer.com/sites/pahweb/ar/es/Productos/Paginas/CIDR.aspx

46. Poncelet D. 2001. Production of alginate beads by emulsification/internal gelation. Annals of the New York Academy of Sciences. 944: 74-82.

47. Prausnitz MR and Langer R. 2008. Transdermal drug delivery. Nature Biotechnology. 26: 1261-1268.

48. Prüße U, Bruske F, Breford J and Vorlop KD. 1998. Improvement of the jet cutting method for the preparation of spherical particles from viscous polymer solutions.

Chemical Engineering & Technology. 21: 153-157.

49. Ragab D, Rohani S, Samaha MW, El-Khawas FM and El-Maradny HA. 2010. Crystallization of progesterone for pulmonary drug delivery. Journal of Pharmaceutical Sciences. 99: 1123-1137.

50. Rajamahendran R, Laguë PC and Baker RD. 1982. Serum progesterone and initiation of ovarian activity in prepuberal heifers treated with progesterone. Canadian Journal of Animal Science. 62: 759-766.

51. Rathbone MJ, Macmillan KL, Bunt CR and Burggraaf S. 1997. Conceptual and commercially available intravaginal veterinary drug delivery systems. Advanced Drug Delivery Reviews. 28: 363-392.

52. Rathbone MJ, Macmillan KL, Inskeep K, Burggraaf S and Bunt CR. 1998. Fertility regulation in cattle. Journal of Controlled Release. 54: 117-148.

53. Rathbone MJ, Bunt CR and Burggraaf S. 2002. US Patent No. 6423039 B1.

54. Rathnam G, Narayanan N and Ilavarasan R. 2008. Carbopol-based gels for nasal delivery of progesterone. AAPS PharmSciTech. 9: 1078-1082.

55. Rekwot PI, Ogwu D, Oyedipe EO and Sekoni VO. 2001. The role of pheromones and biostimulation in animal reproduction. Animal Reproduction Science. 65: 157-170.

56. Roche JF. 1976. Retention rate in cows and heifers of intravaginal silastic coils impregnated with progesterone. Journal of Reproduction and Fertility. 46: 253-255.

57. Scanlon PF, Neville WJ, Burgess TD and Macpherson JW. 1971. Synchronization of estrus in cattle by intravaginal application of progesterone with estrogen administration. Canadian Journal of Animal Science. 51: 250-251.

58. Senuma Y, Lowe C, Zweifel Y, Hilborn JG and Marison I. 2000. Alginate hydrogel microspheres and microcapsules prepared by spinning disk atomization. Biotechnology and Bioengineering. 67: 616-622.

59. Shah NH, Railkar AS, Chen FC, Tarantino R, Kumar S, Murjani M, Palmer D, Infeld MH and Malick AW. 1993. A biodegradable injectable implant for delivering micro and macromolecules using poly (lactic-co-glycolic) acid (PLGA) copolymers. Journal of Controlled Release. 27: 139-147.

60. Shimizu H, Toyoda Y, Takeuchi S, Kawai T and Adachi S. 1967. Synchronization of oestrus and subsequent fertility of beef cattle following the intravaginal administration of gestagen. Journal of Reproduction and Fertility. 13: 555-558.

61. Shively ML, Coonts BA, Renner WD, Southard JL and Bennett AT. 1995. Physico-chemical characterization of a polymeric injectable implant delivery system. Journal of Controlled Release. 33: 237-243.

62. Short RE, Bellows RA, Carr JB, Staigmiller RB and Randel RD. 1976. Induced or synchronized puberty in heifers. Journal of Animal Science. 43: 1254-1258.

63. Siegel RA, Falamarzian M, Firestone BA and Moxley BC. 1988. pH-Controlled release from hydrophobic/polyelectrolyte copolymer hydrogels. Journal of Controlled Release. 8: 179-182.

64. Thibier M and Wagner HG. 2002. World statistics for artificial insemination in cattle. Livestock Production Science. 74: 203-212.

65. Tomoda K, Watanabe A, Suzuki K, Inagi T, Terada H and Makino K. 2012. Enhanced transdermal permeability of estradiol using combination of PLGA nanoparticles system and iontophoresis. Colloids and Surfaces B: Biointerfaces. 97: 84-89.

66. Tsung J and Burgess DJ. 2012. Biodegradable Polymers in Drug Delivery Systems. In

Page39

Fundamentals and applications of controlled release drug delivery (eds. J Siepmann, RA Siegel and MJ Rathbone), pp. 107-123. Springer, New York.

67. Valenta C and Auner BG. 2004. The use of polymers for dermal and transdermal delivery. European Journal of Pharmaceutics and Biopharmaceutics. 58: 279-289.

68. Van den Berg MP, Verhoef JC, Romeijn SG and Merkus FWHM. 2004. Uptake of estradiol or progesterone into the CSF following intranasal and intravenous delivery in rats. European Journal of Pharmaceutics and Biopharmaceutics. 58: 131-135.

69. Viegas TX, Reeve LE and Henry RL. 1994. US Patent No. 5318780.

70. Villeneuve AM. 2001. How to stimulate your partner. Science. 291: 2099-2101.

71. Whitten W. 1999. Pheromones and regulation of ovulation. Nature. 401: 232-233.

72. Zhao J, Lahiri-Chatterjee M, Sharma Y and Agarwal R. 2000. Inhibitory effect of a flavonoid antioxidant silymarin on benzoyl peroxide-induced tumor promotion, oxidative stress and inflammatory responses in SENCAR mouse skin. Carcinogenesis. 21: 811-816.

Page 40