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Article · January 2017

DOI: 10.22192/lsa.2017.3.4.6

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Life Science Archives (LSA)

ISSN: 2454-1354

Volume – 3; Issue - 4; Year – 2017; Page: 1130 – 1137

DOI: 10.22192/lsa.2017.3.4.6

Research Article

HORMONAL DELIVERY PGF 2A WITH CHITOSAN POLYMER TO TREATMENT INFERTILITY COW

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Abstract

The present study conducted preparing type of Chitosan from shrimp with the ability to biodegradation and compatibility in tissue. The new mesh of Chitosan were characterized by IR spectroscopy to identification of functional groups and then mixed with PGF2a after dissolve with ethanol alcohol. The aim of this study was to determine the effect of hormonal delivery (PGF2a) to treated the infertility in cows. Through induce estrus, pregnancy diagnosis by ultrasound to conform the fetus and blood sample to evaluate the level of progesterone hormone. Ten cows Holstein Fersion breed, aged (4 - 5 years old) and weight (350 – 400 kg) were used in this study. They divided randomly into two equal groups each group contain five cows, Group (A) give intramuscular injection with PGF2a hormone mix with chitosan powder after dissolve in ethanol (150 mg) at 11 day as in followings Option -1, while Group (B) with PgGF2a hormone mix with chitosan powder that dissolve in ethanol (150 mg) at 5 day as in followings Option -11. Cows were examined by ultrasonography every month to confirm presence of pregnancy and rectal palpation to confirm pregnant. The result shows that animal response to estrus in two groups were 100 % with duration of response 21.6±11.80 while estrus response in Group (B) 21.12±12.50, the pregnancy rate recorded in two groups 100 %, and parturition was high percentage 100 %. The conclusion of the hormonal delivery (PGF2a with chitosan) was successful for treatment infertility in cow suffered from repeated estrous or cystic ovary through high percentage of pregnant and calving.

Article History

Received : 05.06.2017

Revised : 02.07.2017

Accepted: 30.07.2017

Key words: Chitin structure, Chitosan structure, Chitosan derivatives, Biomaterials, PGF2a, Infertility, Cattle and Hormonal delivery.

1. Introduction

Bovine gonadal hypoplasia is not easy to diagnose and in cases of bilateral ovarian hypoplasia heifers do not develop secondary sexual characteristics. They are anoestrus and infertile. Where the condition is unilateral, normal sexual organs and oestrous activity may be observed. Such animals are fertile, although less so than normal. The condition is potentiated by an autosomal recessive gene with incomplete penetrance, and therefore the incidence of gonadal hypoplasia can be

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LSA ISSN: 2454-1354

reduced by using only animals (both male and female) with normally developed sexual organs as breeding stock (Arkema *et al*., 1992). Prostaglandins PGE2 probably increase the chance of vaginal delivery in 24 hours, they increase uterine hyperstimulation with fetal heart changes but do not effect or may reduce caesarean section rates. They increase the likelihood of cervical change, with no increase in operative delivery rates. PGE2 tablets, gels and pessaries appear to be as effective as each othe, any differences between formulations are marginal but may be important (Anna *et al*., 2014).

Drug delivery refers to approaches, formulations, technologies, and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effect, it may involve scientific site-targeting within the body, or it might involve facilitating systemic pharmacokinetics. In any case, it is typically concerned with both quantity and duration of drug presence. Drug delivery is often approached via a drug's chemical formulation, but it may also involve medical devices or drug-device combination products. Drug delivery is a concept heavily integrated with dosage form and route of administration, the latter sometimes even being considered part of the definition (Wangand Von Recum, 2011).

Drug delivery technologies modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance. Drug release is from: diffusion, degradation, swelling, and affinity-based mechanisms. Most common routes of administration include the preferred non-invasive peroral (through the mouth), topical (skin), transmucosal (nasal, buccal/sublingual, vaginal, ocular and rectal) and inhalation routes. Many medications such as peptide and protein, antibody, vaccine and gene based drugs, in general may not be delivered using these routes because they might be susceptible to enzymatic degradation or cannot be absorbed into the systemic circulation efficiently due to molecular size and charge issues to be therapeutically effective. For this reason many protein and peptide drugs have to be delivered by injection or a nanoneedle array. For example, many immunizations are based on the delivery of protein drugs and are often done by injection (Bertrand and Leroux, 2011).

Chitin is the second most important natural polymer in the world. The main sources exploited are two marine crustaceans, shrimp and crabs. Our objective is to appraise the state of the art concerning this polysaccharide: its morphology in the native solid state, methods of identification and characterization and chemical modifications, as well as the difficulties in utilizing and processing it for selected applications. Chitosan, which is soluble in acidic aqueous media, is used in many applications (food, cosmetics, biomedical and pharmaceutical applications). We briefly describe the chemical modifications of Chitosan - an area in which a variety of syntheses have been proposed tentatively, but are not yet developed on an industrial scale. This review emphasizes recent papers on the high value-added applications of these materials in medicine and cosmetics (Marguerite, 2006; Islem and Marguerite, 2015).

Chitin or poly (β-(1→4)-N-acetyl-d-glucosamine) is a natural polysaccharide of major importance. This biopolymer was synthesized by enormous number of living organisms and it belongs to the most abundant natural polymers, after cellulose. In the native state, chitin occurs as ordered crystalline microfibrils which form structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast. So far, the main commercial sources of chitin are crab and shrimp shells. In industrial processing, chitin is extracted by acid treatment to dissolve the calcium carbonate followed by alkaline solution to dissolve proteins. In addition, a decolorization step is often added in order to remove pigments and obtain a colorless pure chitin (Rinaudo, 2006). The aim of this study is to determine the effects of hormonal delivery of prostaglandins to control infertility in cow.

2. Material and Methods

Chitosan Preparation

The term chitosan usually refers to a family of polymers obtained after chitin deacetylation to varying degrees. In fact, the acetylation degree, which reflects the balance between the two types of residues, differentiates chitin from chitosan. When the DA (expressed as molar percentage) is lower than 50 mol %, the product is named chitosan and becomes soluble in acidic aqueous solutions. During deacetylation, acetyl groups are removed but also depolymerization reaction occurs, indicated by changes in MW of chitosan, Chitin can be converted to chitosan by enzymatic preparations or chemical process. Chemical methods are used extensively for commercial purpose of chitosan preparation because of their low cost and suitability to mass production. From a chemical point of view, either acids or alkalis can be used to deacetylate chitin. However, glycosidic bonds are very susceptible to acid; therefore, deacetylation is used more frequentlyThe N-deacetylation of chitin is either performed heterogeneously, or homogeneously. Commonly, in the heterogeneous method, chitin is treated with a hot concentrated solution of NaOH during few hours, and chitosan is produced as an insoluble residue deacetylated up to ~85 % – 99 %. According to the homogeneous method, alkali chitin is prepared after dispersion of chitin in concentrated NaOH (30 g NaOH/45 g H2O/ 3 g Chitin) at 25 °C for 3 hrs or more, followed by dissolution in crushed ice around 0 °C. This method results in a soluble chitosan with an average degree of acetylation of 48 % – 55 %.

This process produces deacetylation with acetyl groups uniformly distributed along the chains, for example chitosan with DA = 10 % after 580 hrs at 25 °C the solubility of chitosan can be characterized not only by the fraction of 2-acetamido-2-deoxy-d-glucose units in the molecule but also by the N-acetyl group distribution. The deacetylation reaction performed under heterogeneous conditions gives an irregular distribution of N-acetyl-d-glucosamine and d-glucosamine residues with some blockwise acetyl group distribution along polymeric chains. Thus, solubility and degree of aggregation of chitosan can vary in aqueous solutions leading to changes in their average characteristics. For instance, physico-chemical properties of such chitosan's may differ from those of randomly acetylated chitosans obtained under homogeneous conditions. Furthermore, variations in chitosan preparation may also result in changes of: DA, distribution of acetyl groups along the chains, MW and viscosity in solution.

In fact, many parameters in the deacetylation reaction can impact the characteristics of the final chitosan, the investigated the effect of temperature, processing time and mechanical shear on chitosan characteristics, and found that temperature and processing time have a significant effect on DA and MW. The chitosan DA is greatly affected by temperature and repetition of alkaline steps. The effects of time and NaOH concentration also examined the effect of time reaction and temperature. All these studies were conducted using a classical one variable at a time experimentation. This indicates that MW and DA of chitosan are mainly affected by NaOH concentration, reaction time, temperature and repetition of alkaline steps. Additional factors such as reaction reagent, atmosphere, particle size, chitin and solvent ratio, and source of raw material were also tested in others studies attempted to optimize chitin deacetylation by response surface methodology (controlling MW or DA) using temperature and reaction time variables. The studied the effect of temperature, time and NaOH concentration on the deacetylation. The influence of NaOH concentration, temperature and solution/chitin ratio and found that chitosan DA was decreasing with increase of temperature and NaOH concentration. Other parameters, such as: the use of alkali successive baths, atmospheric conditions and presence of different additives could influence deacetylation but were not considered previously in optimization studies Deacetylation was investigated using seven factors: the alkali reagent, its concentration, temperature, reaction time, the use of successive baths, atmospheric conditions and the use of sodium borohydride, a reducing agent. For that

purpose, a fractional factorial design was applied and a mathematical model was established to allow optimizing experimental conditions for chitosan of desired DA. Results clearly revealed a significant effect of temperature and the alkali reagent nature (NaOH treatment is much more efficient than KOH.

Study animals

This study was carried out on 10 healthy cow (Frisian Holstein) range in age from 4 - 5 years old and weight 350 - 400 kg. The animals were housed semi opened, in animal place at Surgical and obstetric department of the college veterinary medicine Basra university. All experimental animals were undergone to a program of vaccination as following against enterotoxaemia, also deformed with cur fluke (Ireland) via oral route against liver fluke and g Experimental design. The animals were exposed to the same environment including climate management and feeding for one month (before starting experiment) to acclimatize and adopt them to the place. The cows were divided randomly to Two Groups: A and B, each group includes 5 cow. They were submitted to trans - abdominal ultrasonography to ensure that cow were not pregnant, free from any complications.

Group - A: Include (5 cow) I/M Injected with PGF2a hormone mix with chitosan powder that dissolve in ethanol (150 mg) at 11 day as in followings Option - 1.

Group - B: Include (5 cow) I/M injected with PgGF2a hormone mix with chitosan powder that dissolve in ethanol (150 mg) at 5 day as in followings Option -11.

Estrous synchronization

Products based on Prostaglandin F2a: Products available for this type of program include the following the PGF2a use With each of these products remember that the compound causes Luteolysis (breakdown of the corpus luteum) which then causes progesterone production to drop. This then reduces the effect of progesterone. The Programs as with all synchronizing programs, advanced planning was required (Table – 1).

I Option: Begin this type of program by injecting cycling females with PGF2a at 11 day intervals breed in one of two ways; inseminate all cows between 72 and 80 hours after the second injection without regard for estrus and inseminate each cow at 12 hours after detected estrus.

II Option 2: As inject PGF2a and breed cows detected in estrus during the subsequent 5 day period and cows not detected during this period will receive a second PGF2a injection 11 days after the first and should be bred at a fixed time or on detection as discussed previously by Islam (2011).

Ultrasonographic examinations were conducted using a convex 7.5 MH2 transabdominal (4.0 cm length) 7.5 transducer was well lubricated with (carboxy methyl-cellulose contact gel) was applied to the test side, area of 150 - 200 cm^2 on the right flank above and under after removing the hairs over it. Then, the transducer was placed at the right side of the cow 5.0 cm in front of the rear leg and 2.5 cm above the teat. Pregnant and non-pregnant cows were determined using real- time monitor by detection of foetal-heart, spinal cord, head, limbs, foetal organ and other structures. Ultrasonography examination (abdominal) was done in a special room, using special gel (coupling) for the probe and the examination (ventro-lateral area) area was shaved and disinfected carefully. The interval period to examination is done on 30, and then every month .The animal was standing position. Duration of response the period initiated from the end of the treatment to estrus behavior was shown animals were under supervision after treatment for detecting the estrus behavior and natural mating to record the ratio of response to hormonal treatment. Successful pregnancy was recorded at the end of calving period.

Blood samples Collection and Processing

Blood samples were collected by disposable syringes from jugular vein after shaving and disinfecting the area. Blood was collected to estimate the concentration of progesterone in the peripheral blood circulation. The collected blood samples (5 - 8 ml) were

immediately stored in cold box and transported to laboratory where the serum was separated by centrifugation at 3000 rpm for 15 min and then, stored in plastic tubes at 20 °C until assayed at the laboratory. The samples collection was done in the gestation (late stage) and then 20 day after parturition.

The principles of the Hormonal test - Competitive enzyme immunoassay

The antibody, native antigen, and enzyme antigen coagulate are the essential reagents required for an enzyme immunoassay. Depending on the mixing of the serum which contains the native antigen, with the biotinylated antibody, and the enzyme – antigen coagulate. So, according to that mixing, a result of competition reaction will occur between the enzyme – antigen conjugate and the native antigen for a limited number of antibody binding sites. Progesterone concentration measurement in cow, the measurement of progesterone was done by using progesterone enzyme immunoassay test kit. The origin of the kit is Moonblind Inc. in USA.

4. Results

Estrous synchronizations

The results describe the type of treatment, animal response, duration of response and pregnancy rate in cow. Cow response to estrus in Group - A that receive (Chitosan + PGF2a) is 100 % with duration of response at 11 day intervals breed in one of two ways; inseminate all cows between 72 and 80 hours after the second injection, while Group – B that receive inject PGF2a and breed cows detected in estrus during the subsequent 5 day period and cows not detected during this period will receive a second PGF2a injection 11 days after the first does in estrous always seek male finally accept the artificial insemination (Table – 1).

Progesterone Concentration

The results explains the mean and Stander Deviation of serum progesterone concentration in late pregnancy in cow recorded in Group - B was higher than Group - A, Fig - 1, Serum progesterone concentration in day 20 after parturation, Group - B was higher than Group – A. Progesterone levels remain high through gestation. testing progesterone levels at 19 - 24 days after insemination will indicate pregnancy if the progesterone level was high. However, confirmation of pregnancy by palpation was required and ultrasound examination (Fig - 2 and 3). The females having the levels under this value were considered non-pregnant. The ones having the above mentioned values higher than 3 ng/ml were considered pregnant.

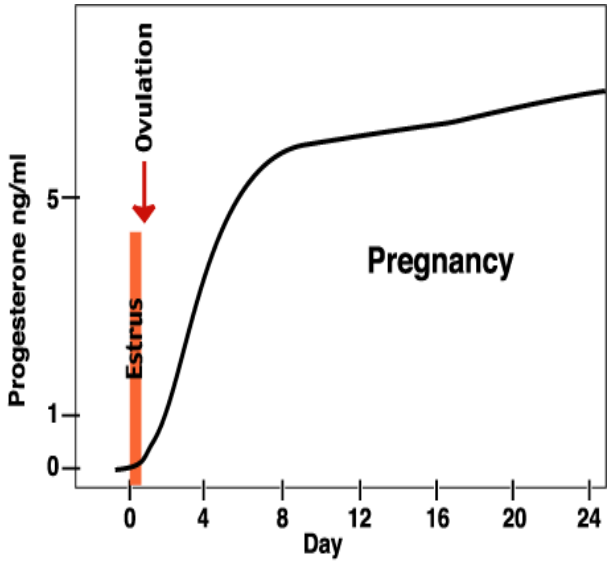

Figure - 1: Diagram explain the level of progesterone in pregnancy cow

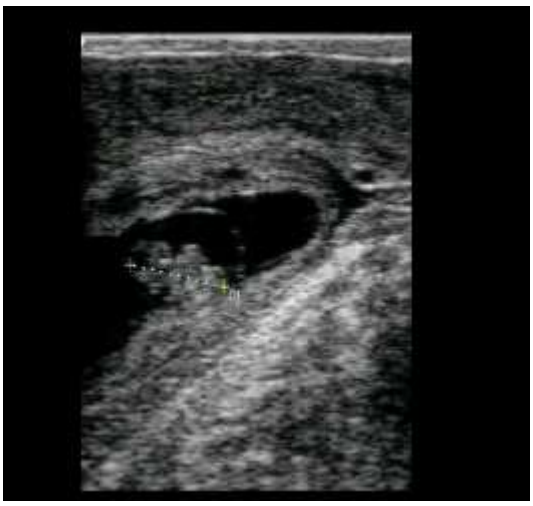

Figure – 2: Image of uterus of cow on day 30 of aminion pregnancy surrounds the Embryo as a thin hyperechoic line (Group – A)

Table - 1: The type of treatment, animals response, duration of response and pregnancy rate in cow

Groups	No. of goats	Type of treatment	Animals response (estrus show)		Duration of response M ±SE hrs	Pregnancy rate	
			No.	%		No.	%
GA	5	Chitosan + PGF2a 150 mg at 11 day as in followings Option -1.	5	100	21.6±11.80 b*	5	100 b*
GB	5	Chitosan+PGF2a 150 mg- at 5 day as in followings Option -11	5	100	21.6±12.50 a*	5	100 b*

*Different small letters mean significance differences ($p<0.01$).

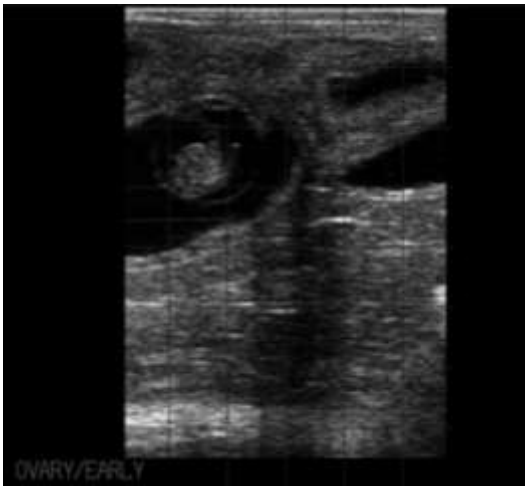

Figure – 3: Image of uterus of cow in 30 day of the gestation (Group – B)

4. Discussion

Estrus synchronization plays a major role in Fixed time breeding, Artificial insemination (AI) and Embryo transfer (ET) (Jainudeen *et al*., 2000). There are number of synchronizing methods for goats, the most common administration of progestagen application in goats *via* intravaginal sponge, (Bladasarre and Karatzas, 2004), the most widely used procedure for synchronizing of estrus are 12 – 21 days of fluorogestone acetate (FGA) or medroxy progesterone acetate (MAP) impregnated intravaginal sponge treatment (Whitly and Jackson, 2004; Lionel, 2007) and intramuscular injection of pregnant mare serum gonadotrophin (PMSG) at progestagen with drawal.

Early diagnosis of pregnancy and fetal sexing using ultrasonography enhances the reproductive management on the farm and improves the commerce of pregnant animals (Santos *et al*., 2004; Robert and Walter, 2007) was important in livestock production to make culling or rebreeding decision for food allotment and for clinical and research purposes. Examination of the goat for pregnancy may done as apart of are productive herd health program. The overall increase in progesterone levels during gestation and decline towards the pre-partum observe in this study also similar to those recorded by Ozpinar and Firat (2003). However, the encapsulation of a given lipophilic active molecule into nanoemulsion droplets for their homogeneous dispersion in water has been shown to strongly depend on the physicochemical properties (and thus on the nature) of the excipients used. Indeed, it depends on the solubilization of such active molecules in the oil used in the nanoemulsion formulation, potentially with the help of a cosolvent. Accordingly, the scientific approach chosen consists of adapting the low - energy nanoemulsification process to the molecule to be encapsulated, involving the use of a cosolvent for enhancing drug solubilization (Michael *et al*., 2000).

Ultrasonography is a non-invasive and it plays valuable roles in diagnosis of various physiological and pathological conditions of the reproductive organs of ruminants (Dimitrov *et al*., 2002; Kahn, 2004). There are two key conditions

in the nanoemulsification process: (1) The drugs solubilization in the organic phase once this solubilization is achieved and (2) This drug containing organic phase must still induce the spontaneous emulsification process. These 2 points are thoroughly investigated in the present study. However, the main difficulty arises in the first point because the self-emulsification itself is directly linked to the nature of the oil - nonionic surfactant couple used (and their respective affinities). In this way, adapting the processes in the encapsulation of guest molecules will mainly involve the choice of these latter molecules for the oil - nonionic surfactant couples available. Additional solubilizing substances, which can be a cosolvent, can also be used in the organic phase to enable the drug to be incorporated into oil (Barbe *et al*., 2004).

Increased PI hydrolysis stimulated by OT was also dependent on reproductive status because OT stimulated PI hydrolysis in the endometria of cyclic, but not pregnant, cows. Similar results were obtained for cows at both locations as was indicated by the lack of an interaction of location and reproductive status treatment, or the interaction of reproductive status and treatment. This pattern of PI hydrolysis was similar to patterns of PGF2a secretion stimulated by OT that have been reported for cyclic and pregnant cows and ewe and was similar to the pattern of PI hydrolysis that was reported for ewes. The reduced responsiveness of endometria to OT in pregnant cows probably resulted from the inhibition of the expression of endometrial OT receptors by trophoblast interferon-t previous report that peripheral progesterone concentration did not influence the PGF2a secretory response to OT on d 18.5 to 19.5 postestrus (Tysseling *et al*., 1998). The conclusion of the hormonal delivery (PGF2a with chitosan) was very successful for treatment infertility in cow suffer from repeated estrous or cystic ovary through high percentage of pregnant and calving.

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DOI Number DOI: 10.22192/lsa.2017.3.4.6

How to Cite this Article:

Abdulbari A. Alfaris, Basil A. Abbas, Shsker A. Neama Aljadaan and Salah Sh. Al-Luaibai. 2017. Hormonal Delivery PGF 2A with Chitosan polymer to treatment infertility cow. *Life Science Archives*, 3 (4): 1030 – 1037.

DOI: 10.22192/lsa.2017.3.4.6