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DRUGS DELIVERY TREATED UNILATERAL OVARIAN HYPOPLASIA AND REPEAT BREEDER COWS

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Abstract

The present study was conducted for preparing type of chitosan polymer mixed with PGF2a after dissolve with ethanol alcohol. Ten cows of Holstein Fersion breed, aged (4 - 5) years, 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 mixed with chitosan powder after dissolve in ethanol (150 mg) at 11 day, while Group (B) with PgGF2a mixed with chitosan powder that dissolve in ethanol (150 mg) at 5 day. Cows were examined by ultrasonography every month to confirm presence of pregnancy and rectal palpation to confirm pregnancy. 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 is high percentage 100 %. The conclusion of the hormonal delivery (PGF2a with chitosan) was successful for treatment infertility in cow suffers from unilateral ovarian hypoplasia and repeat breeder cows through high percentage of pregnant and calving.

Key words: Chitin structure, Chitosan structure, Biomaterials, PGF2a, Unilateral ovarian hypoplasia and Repeat breeder cows.

1. Introduction

Bovine gonadal hypoplasia is not easy to diagnose and in cases of bilateral hypoplasia ovarian heifers do not develop secondary sexual characteristics. They are sterile where the condition is unilateral, normal sexual behaviour and oestrous activity may be observed. Such animals are half 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 reduced by using only animals (both male and female) with normally developed sexual organs as breeding stock (Arkema *et al*., 1992). Prostaglandins PGf2a probably increase the chance of vaginal delivery in 24 hours, they increase uterine hyper stimulation 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 other, any differences between formulations are marginal but may be important (Anna *et al*., 2014).

Drug delivery technologies modify drug release profile, absorption, distribution and elimination for the benefit of improving product

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efficacy and safety, a human concept. Drug release is from diffusion, degradation, swelling and affinity-based mechanisms. Many medications such as peptide and protein, antibody, vaccine and gene based drugs, in general may not be delivered using *via* mouth, eye, skin, nasal 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). In recent years, more studies have been done on synthesis and swelling behavior of hydrogels *via* free radical polymerization and cross linking in the presence of an initiator and a cross linking agent. Super absorbent hydrogels have more applications in the field of hygienic products (Hassan and Zahra, 2014).

Chitin is the second most important natural polymer in the world. The main sources exploited are two marine crustaceans, shrimp and crabs. 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 (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 is 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 with chitosan to control infertility in cows suffering from unilateral hypoplasia.

2. Materials and Methods

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 frequently. The 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 H_2O/ 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 % (Barbe *et al*., 2004). 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 block wise acetyl group distribution along polymeric chains. Thus, solubility and the 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 (Wangand von Recum, 2011).

Animal Study

The animal study was carried out on 10 healthy cow of Frisian Holstein breed, their age ranged from 4 - 5 years and the weight 350 - 400 kg. The animals were housed in semi opened system presented in Surgery and obstetric department of the college veterinary medicine Basra University. Suffering from unilateral ovarian hypoplasia 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.

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 contained five cow. They were examined with Trans - Abdominal Ultrasonography to ensure that cow was not pregnant, free from any complications.

Group A: It includes 5 cow I/M Injected with PGF2a hormone mixed with chitosan powder that dissolve in ethanol (150 mg) at 11 day as in followings. 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.

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

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 is required (Table – 1).

Ultra-sonographic examinations were conducted using a convex 7.5 MH2 transabdominal (4.0 cm lenght) 7.on day 30 and every month in standing position. 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 .Successful pregnancy was recorded at the end of calving period.

Blood samples were collected with disposable syringes from jugular vein 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 r/m for 15 min and then, stored in plastic tubes at 20 °C until assayed at the laboratory. The samples were collected at early mid, late stage of gestation and then 20 days after parturition.

The principles of the hormonal test used Competitive enzyme binding immunoassay. The test were done by 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 (Moonblind Inc. in USA).

3. Results

Estrous synchronizations

The results explains the mean and Stander Deviation of serum progesterone concentration in late pregnancy in cow recorded

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

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

Groups	No. of goats	Type of treatment	Animals response (estrus show) (No. & %)	Duration of response (M±SE hrs)	Pregnancy rate (No. %)
Basrah	5	Chitosan + PGF2a 150 mg at 11 day	5 & 100	21.6±11.80b*	5 & 100b*
Basrah	5	Chitosan + PGF2a 150 mg at 5 day	5 & 100	21.6±12.50a*	5 & 100b*

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

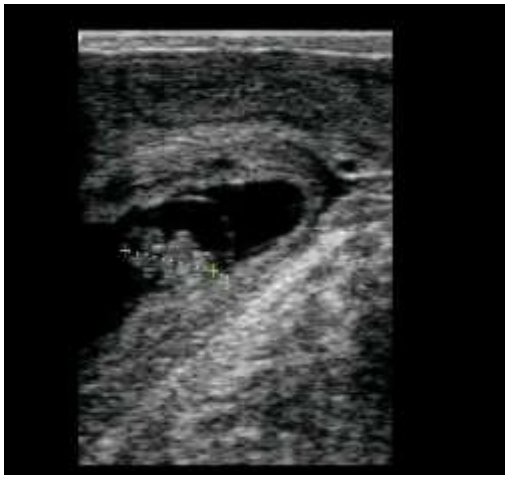

Figure – 1: Image of uterus of cow on Day 30 of Amnion pregnancy surrounds the Embryo as a thin Hypercholic line (Group – A)

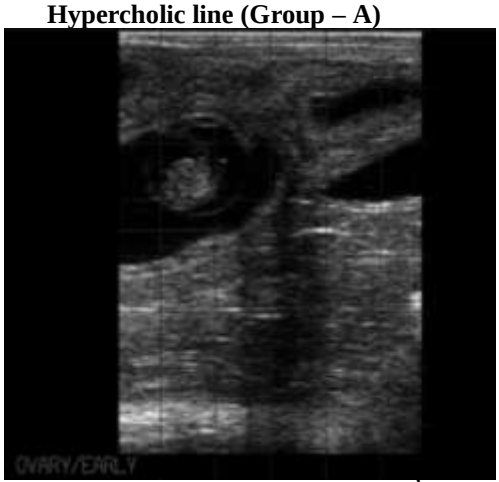

Figure – 2: Image of Uterus of cow in 30th day of the generation (Group - B)

4. Discussion

Recent improvements in our understanding of methods of inducing and synchronizing estrus and ovulation in postpartum beef cows and replacement beef heifers creates the opportunity to significantly expand the use of artificial insemination in both purebred and commercial herds. Technology now exists to successfully inseminate beef cows at predetermined fixed times with pregnancy rates comparable to those achieved with heat detection While many options exist for synchronization of estrus and ovulation, this short list of protocols was developed based on available research data and field use by the Beef Cattle Reproduction Leadership Team. This group is composed of representatives from the AI and pharmaceutical industries veterinarians, and reproductive physiologists from the Beef Reproduction Task Force with active research programs in this area.

Estrus synchronization plays a major role in fixed time breeding, artificial insemination (AI) and embryo transfer (ET) (Jainudeen *et al*., 2000). A number of synchronizing methods for cows, the most common administration of progestagen application in goats is *via* intravaginal sponge, 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 (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) is important in livestock production to make culling /rebreeding decision for food allotment and for clinical and research purposes. 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, (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 (Barbé *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 the 18.5 to 19.5 postestrus (Tysseling *et al.*, 1998). Improve the understanding of methods to assess male fertility and how it affects the success of AI programs.

Carrier-mediated drug delivery has emerged as a powerful methodology for the treatment of various pathologies. The therapeutic index of traditional and novel drugs is enhanced *via* the increase of specificity due to targeting of drugs to a particular tissue, cell or intracellular compartment, the control over release kinetics, the protection of the active agent or a combination of the above. Polymer composites were proposed as drug carriers over 30 years ago and have received growing attention since mainly due to their stability, enhanced loading capabilities and control over physicochemical properties. In addition to systemic administration, localized drug release may be achieved using macroscopic drug depots close to the target site. Among various systems considered for this approach, *In situ* - forming

biomaterials in response to environmental stimuli have gained considerable attention, due to then on-invasive character, reduction of side effects associated with systemic administration and better control over biodistribution (Sahoo *et al.*, 2009). The conclusion of the hormonal delivery (PGF2a with chitosan) is very successful for treatment infertility in cow suffer from unilateral ovarion hypoplasia and repate ebreeder cows through high percentage of pregnant and calving.

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