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Research Article

Entrapment Efficiency of Novel Chitosan Nanoconjugated GnRH for Estrus Synchronization in Kilakarsal Ewes

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ABSTRACT

The current research work was undertaken to induce estrus synchronization in Kilakarsal ewes, using chitosan nanoconjugated GnRH and to assess its efficacy and economical feasibility under semi intensive farming conditions The total GnRH (16.05 µg/mL) and free GnRH (2.80 µg/mL) contents as obtained by their respective protein values and entrapment efficacy was calculated and found to be 82.55 %. The nanoparticles prepared in this study showed the highest EE value of 82.55%. As EE values represent the conjugation abilities of GnRH with chitosan, it may be inferred that the Chitosan nanoconjugated GnRH may be the most suitable and cost effective treatment for ES in ewes.

Keywords: *Estrus synchronization, Nano particls, GnRH, Ewes*

INTRODUCTION

One of the largest factors having an impact today's sheep industry that will ensure ita future success and long-term sustainability is reproductive performance. To produce lambs consistently throughout the year inan accelerated lambing sysem, the use of exogenous hormones Cn be utilized in order to induce a synchronized estru and stimulate cyclicity during the anestrus period. This can be achieved by either lengthening or reducing the duration of the estrus cycle by administration of exogenous progesterone (P4) or prostaglandin (PG) Gonadotropin-releasing hormone (GnRH) may also be incorporated into treatment protocols to assist in initiating hormonal events necessary to synchronize estrous cycles, such as estrus and ovulation, especially in anestrous ewes (Wildeus, 2000).

Longer lasting surges in Gonodotropin hormone (GtH) blood levels can be attained by using multiple injections of GnRH or analogues, which unavoidably involves repeated handling of animals resulting in increased cost, time, labor and avoidable stress to both the animals and handler.

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Another approach which has been successfully employed to provide long lasting surges of GtH in blood was to use sustained release delivery system by implantation method. However, since GnRH having very short half-life undergo proteolysis within a few minutes (Casper, 2015), the newer nanotechnology has come into place for effective delivery of hormones. Therefore, the following research focuses on synchronizing estrus in ewes during both the breeding and non-breeding seasons by potentially reducing the days to detected estrus, while increasing overall ewe prolificacy through the utilization of novel Chitosan Nanoconjugated GnRH.

MATERIALS AND METHODS

Chemicals and Reagents

Gonadotropin Releasing Hormone

Gonadotropin Releasing hormone (GnRH) (amino acid sequence: Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) was procured from Sigma-Aldrich, USA.was used for the preparation of the chitosan nanoconjugated GnRH.

Procedure

High pressure homogenization process was used for conjugation of the nanoparticles, as per the method of Rather et al. (2013). A stock solution 1000µg/mL of GnRH was prepared with water. The stock solution was then added to the chitosan nano particles solution in accordance with 1:4 (drug: carrier) ratio. After homogenization process the resulting mixture was subjected to Bradford assay to determine the total GnRH content, by measurig the protein concentration in the solution. The solution was then kept overnight in the refrigerator at 4°C. Next day, the solution was centrifuged at 2000 rpm and the supernatant collected was again subjected to Bradford assay to determine the free GnRH concentration (mg/mL)

The entrapment efficiency (EE) of the GnRH with chitosan was calculated using the formula:

$$EE\ (\%) = \frac{\text{Total GnRH} - \text{Free GnRH in supernatant (µg/mL)}}{\text{Total GnRH (µg/mL)}} \times 100$$

RESULT AND DISCUSSION

The Chitosan nanoconjugated GnRH solution was centrifuged at 2000 rpm and the supernatant collected was subjected to Bradford assay to determine the free GnRH concentration as mentioned above. The total GnRH (16.05 µg/mL) and free GnRH (2.80 µg/mL) contents as obtained by their respective protein values by Bradford assay. From the values obtained entrapment efficacy was calculated and found to be 82.55 %. Similarly Rather et al. (2013) prepared the chitosan conjugated LHRH nanoparticles based on the ionic gelation method and reported entrapment efficiency of 69 %. Rakhi Kumari et al. (2013) synthesized chitosan nanoencapsulated trypsins based on the ionic gelation method and found entrapment efficiency was ranged from 65 to 75 %.

The main goal in designing nanoparticles as a delivery system is to control particle size, surface properties and release of pharmacologically active agents in order to attain the site-specific action of the drug at therapeutically optimal rate and dose regimen (Vila et al., 2002; Mu and Feng, 2003).

The nanoparticles of zidovudine prepared using chitosan and TPP showed the entrapment efficiency of 52 to 63 % (Adlin & Anton, 2012).

Ferosekhan et al. (2014) synthesized RNA-loaded chitosan NPs based on the ionic gelation method and found that the entrapment efficiency was ranged from 57 to 73 %. Othayoth et al. (2013) reported that chitosan-pluronic polymeric nanoparticles prepared by ionic gelation method showed entrapment efficiency ranged from 47.15 to 58.34 %.

Among the references cited only the values of 69% and 65-75% were reported by Rather et al. (2013) and Rakhi Kumari et al. (2013), respectively, that came closer to the

EE of 82.55 % observed in this study. The other authors had quoted much lower EE%.

Lowest particle size, high PDI of the Chitosan nanoconjugated GnRH prepared by ionic gelation with high pressure homogenization in this study together may have contributed to the better entrapment efficiency observed.

As such EE values represent the conjugation abilities of GnRH preparation with chitosan. Since the nanoparticles prepared in this study showed the highest EE value of 82.55%, it is inferred that the Chitosan nanoconjugated GnRH may be the most suitable and cost effective treatment for ES in ewes.

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