

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.902.005>

## Comparative therapeutic efficacy of Gonadotropin Releasing Hormone (GnRH), Human Chorionic Gonadotropin (HCG) analogues and progesterone in non-infectious repeat breeding crossbred cows

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### ABSTRACT

The present study was conducted on 36 crossbred cows with history of repeat insemination at different intervals, randomly categorized in infectious and non-infectious groups on basis of positive or negative reaction to Whiteside test belonging to the Dairy farm of College of Veterinary science and Animal Husbandry, Mhow and clinical cases of progressive farmers brought for artificial insemination to teaching veterinary clinical complex and at the doorstep of farmers in nearby villages. These selected animals divided into 6 groups GnRH analogue (Gp I), GnRH + HCG (Gp II), HCG alone (Gp III), HCG + GnRH (Gp IV), Progesterone (Gp V) and Control Group (Gp VI). He was found higher conception rate in GnRH + HCG (Gp II), HCG + HCG (Gp ) and Progesterone was 66.66 (4/6), whereas GnRH analogue (Gp I), HCG alone (Gp II) and control group was 50 %.

#### Keywords

Crossbred cows, repeat insemination, GnRH analogue, HCG, Progesterone

#### Article Info

##### Accepted:

05 January 2020

##### Available Online:

10 February 2020

### Introduction

Repeat breeding has been defined as failure to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities (Zemjanis, 1980). Oestrus, the most visible phase of the oestrous cycle is

characterized by nervousness, bellowing and mounting, stands to be mounted by another cow, reduced feed intake and milk production. During the pre-implantation phase of embryonic development, direct progesterone supplementations and GnRH / hCG injections are the approaches to improve embryonic

survival in repeat breeder cows (Mann 2002). In dairy cows, luteal insufficiency and lower progesterone concentrations are known as a cause of embryonic mortality and reduce the pregnancy rates during early embryonic development (Howard *et al.*, 2006).

It has been hypothesized that increasing peripheral progesterone concentrations during the diestrus after insemination may improve embryo development and may suppress luteolysis, resulting in reduced embryonic loss.

GnRH/hCG injection causes a predictable release of LH hence, administration of GnRH and/or hCG before, during and post-insemination would more precisely synchronize ovulation with estrus, increased the pregnancy rates and decreased the early embryonic deaths (Das *et al.*, 2007)

### Materials and Methods

On the basis of above examination, the

animals (non-infectious repeat breeder) were assigned into following groups (Table.1)

#### Group 1

Animals in this group (n=6) were treated with GnRH analogue injection buserelin acetate @ 10 µg (2.5 ml) intramuscularly at the time of Artificial Insemination (AI) and it was followed by 12<sup>th</sup> day after AI.

#### Group 2

Animals in this group (n=6) were treated with GnRH analogue injection buserelin acetate @ 10 µg (2.5 ml) intramuscularly at the time of AI and injection hCG @ 1500 IU intramuscularly on 12<sup>th</sup> day after the AI.

#### Group 3

Animals in this group (n=6) were treated with injection hCG @ 1500 IU intramuscularly at the time of AI followed by 12<sup>th</sup> day after the AI.

**Table.1 Grouping of animals**

Groups	No. of Animals	Type of Animal	Treatment given
Group 1	6	Repeat breeder	GnRH at AI + 12 <sup>th</sup> day
Group 2	6	Repeat breeder	GnRH at AI + hCG on 12 <sup>th</sup> day
Group 3	6	Repeat breeder	hCG at AI + 12 <sup>th</sup> day
Group 4	6	Repeat breeder	hCG at AI + GnRH on 12 <sup>th</sup> day
Group 5	6	Repeat breeder	P <sub>4</sub> on 4 <sup>th</sup> day after AI
Group 6 (Control group)	6	Repeat breeder	Normal saline at AI + 12 <sup>th</sup> day

#### **Group 4**

Animals in this group (n=6) were treated with injection hCG @ 1500 IU intramuscularly at the time of AI and GnRH analogue injection buserelin acetate @ 10 mcg (2.5 ml) intramuscularly on 12th day after the AI.

#### **Group 5**

Animals in this group (n=6) were treated with injection hydroxyl progesterone 500 mg I/M on 4<sup>th</sup> day of estrum after first insemination.

#### **Group 6 (Control group)**

Animals in this group (n=6) were non-infectious repeat breeder crossbred cows and treated with injection normal saline (2ml) intramuscularly at the time of AI and on 12<sup>th</sup> day after AI as placebo.

#### **Pregnancy diagnosis**

Cows were examined per-rectum at 45 to 60 days post- AI to confirm pregnancy.

#### **Conception rate**

Therapeutic efficacy of drugs was judged on the basis of per rectal examination for pregnancy diagnosis at day 45 post treatment.

#### **Statistical analysis**

Data analysis was done as per the standard statistical method by application of Factorial Completely Randomized Design (Snedecor and Cochran, 1994).

#### **Results and Discussion**

Thirty Six crossbred cows showing negative reaction to Whiteside test were subjected to different hormonal therapeutic protocols. Before initiating hormonal treatment, owners

of the animals were supplied with multi mineral bolus for PO use, one bolus on alternate day for four times. The selected repeat breeding crossbred cows with average body condition score (BCS) of 2.75 to 3.50, without visible and palpable genital abnormalities were treated once with SC injection of 10 ml Ivermectin and IM injection of 1.0 g Enrofloxacin to check invisible genital infection and also with IM injection of 10 ml inorganic phosphorus and multivitamins AD3E.

In GnRH + GnRH analogue injection treatment protocol, six non-infectious repeat breeder crossbred cows, each were administered with IM Inj. of Buserelin acetate 10 µg (2.5 ml) on the day of diagnosis/ treatment (0<sup>th</sup> day of estrus) followed by second inj. of Buserelin acetate 10 µg (2.5 ml) IM on 12<sup>th</sup> day post- AI.

The conception rate in GnRH + GnRH analogue treated non-infectious repeat breeder cross bred cows were found to be 16.66 (1/6) per cent at 1<sup>st</sup> oestrus, 33.33 (2/6) per cent in 2<sup>nd</sup> oestrus and 00.00 (0/6) per cent in 3<sup>rd</sup> oestrus, with an overall pregnancy rate of 50.00 (3/6) per cent.

The present findings approximates with the observation reported by Drew and Peters (1992) , who reported 12 per cent higher pregnancy rate in GnRH treated cows on day 12 after insemination than in control animals. An overall enhancement in conception rate of 83.33 as against 33.33 per cent in control groups has been reported in cows through GnRH therapy irrespective of days of administration (Dodamani *et al.*, 2010).

The present results are in agreement with the finding that the use of GnRH at the time of AI increases conception rate in dairy cows (Parmar *et al.*, 2013 and Jaswal and Singh, 2013). Similarly, Lopez-Gaitus *et al.*, 2006

reported that GnRH administration during AI and after 12 days increased the pregnancy rates.

In GnRH analogue + hCG injection treatment protocol, six non-infectious repeat breeder crossbred cows each were then administered with IM Inj. of Buserelin acetate 10 µg (2.5 ml) on the day of diagnosis/ treatment (0<sup>th</sup> day of estrus) followed by second IM Inj. of hCG, 1500 IU on 12<sup>th</sup> day post- AI.

The conception rate in GnRH analogue + hCG injection treated non-infectious repeat breeder cross bred cows were found to be 33.33 (2/6) per cent at 1<sup>st</sup> oestrus, 16.66 (1/6) per cent in 2<sup>nd</sup> oestrus and 16.66(1/6) per cent in 3<sup>rd</sup> oestrus, with an overall pregnancy rate of 66.66 (4/6) per cent.

Administration of GnRH on day 0 post-AI might have induced luteinisation and /or ovulation of dominant follicle of first follicular wave which successfully increased concentrations of progesterone during mid-luteal phase in lactating dairy cows (Beltran and Vasconcelos, 2008) and minimized the luteolytic cascade by endometrial cells during the period of maternal recognition of pregnancy, which favours pregnancy maintenance (Kerbler *et al.*, 1997; Mann *et al.*, 2001) and Nayak (2015).

In hCG injection + hCG injection treatment protocol, six non-infectious repeat breeder crossbred cows each were then administered with IM Inj. of hCG, 1500 IU on the day of diagnosis/ treatment (0<sup>th</sup> day of oestrus) followed by second IM Inj. of hCG, 1500 IU on 12<sup>th</sup> day post- AI.

The conception rate in hCG injection + hCG injection treated non-infectious repeat breeder cross bred cows were found to be 16.66(1/6) per cent at 1<sup>st</sup> oestrus, 33.33 (2/6) per cent in 2<sup>nd</sup> oestrus and 16.66(1/6) per cent in 3<sup>rd</sup>

oestrus, with an overall pregnancy rate of 66.66 (4/6) per cent. The conception rate obtained with hCG treatment group was higher than in control group. The present findings are in agreement Senthil *et al.*, (2014). Although, Parmar *et al.*(2013) and Mathew *et al.*, (2013) reported higher conception rate in hCG treated repeat breeder cows but the present findings differed from Paksoy and Kalkan, 2010, who concluded that hCG had no effect on conception rate if injected at the time of AI.

In hCG injection + GnRH analogue treatment protocol, six non-infectious repeat breeder crossbred cows each were then administered with IM Inj. of hCG, 1500 IU on the day of diagnosis/ treatment (0<sup>th</sup> day of oestrus) followed by second inj. of Buserelin acetate 10 µg (2.5 ml) IM on 12<sup>th</sup> day post- AI.

The conception rate in hCG injection + GnRH analogue treated non-infectious repeat breeder cross bred cows were found to be 16.66 (1/6) per cent at 1<sup>st</sup> oestrus, 16.66 (1/6) per cent in 2<sup>nd</sup> oestrus and 16.66 (1/6) per cent in 3<sup>rd</sup> oestrus, with an overall pregnancy rate of 50.00 (3/6) per cent.

HCG (Human chorionic gonadotropin) injection causes a predictable release of luteotrophic hormone (LH) hence, administration of hCG before, during and post-insemination would more precisely synchronize ovulation with estrus, increased the pregnancy rates and decreased the early embryonic deaths (Das *et al.*, 2007).

The improvement in the conception rate with the use of GnRH during luteal phase has been attributed to the fact that GnRH on day 12 induces ovulation of the first wave dominant follicle (FWDF), thus forming an accessory CL and enhancing progesterone production early in the cycle.

This increase in progesterone secretion caused by GnRH may facilitate embryonic development (Mann and Lamming, 1999). In Progesterone injection treatment protocol, six non-infectious repeat breeding crossbred cows were treated with IM inj. of 500 mg of hydroxyl-progesterone on day 4<sup>th</sup> day of insemination.

The conception rate in Progesterone injection treated non-infectious repeat breeder cross bred cows were found to be 33.33 (2/6) per cent at 1<sup>st</sup> oestrus, 16.66(1/6) per cent in 2<sup>nd</sup> oestrus and 16.66(1/6) per cent in 3<sup>rd</sup> oestrus, respectively, in the treatment cycle itself and post-treatment I and II cycles. The pooled conception rate obtained in treated crossbred cows was 66.66 (4/6) per cent.

The present finding of 70.00 per cent overall conception rate obtained in repeat breeder cows administered with 500 mg progesterone IM on 5thday post-AI was closely in agreement with the previous reports of Srivastava and Kharche (2001); who obtained 65.21 per cent conception rate, respectively. The conception rate recorded in the present study were higher than the conception rate of 44.00, 30.00 and 30.88 per cent recorded by Stevenson and Mee(1991), Singh and Nanda(2007).

In injection normal saline + injection normal saline as Placebo treatment protocol, six non-infectious repeat breeder crossbred cows, each were administered with IM Inj. of normal saline (2ml) on the day of diagnosis/ treatment (0<sup>th</sup> day of oestrus) followed by second inj. of normal saline (2ml) IM as Placebo on 12<sup>th</sup> day post- AI.

The conception rate in injection normal saline + injection normal saline as Placebo treated non-infectious repeat breeder cross bred cows were found to be 16.66 (1/6) per cent at 1<sup>st</sup> oestrus, 16.66 (1/6) per cent in 2<sup>nd</sup> oestrus and

16.66 (1/6) per cent in 3<sup>rd</sup> oestrus, with an overall pregnancy rate of 50.00 (3/6) per cent. In control group cows, the overall conception rate obtained was 50 per cent which closely corroborates with the findings of Bhattacharya and Hafiz(2009);Derar *et al.*(2012) and Patel *et al.*(2014a).

In contrast, relatively lower overall conception rate obtained by others between 40 to 60 per cent include, Singh *et al.*, (2002) However, much lower conception rate below 40 per cent were also observed by others include Patel *et al.*, (2005b); Singh and Nanda ( 2007); Kadarbhai *et al.*(2012)and Biradare *et al.*(2014).

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**How to cite this article:**

Amit Kumar, Sudarshan Kumar, Madhu Shivhare, Ranjit Aich and S. P. Nema. 2020. Comparative therapeutic efficacy of Gonadotropin Releasing Hormone (GnRH), Human Chorionic Gonadotropin (HCG) analogues and progesterone in non-infectious repeat breeding crossbred cows. *Int.J.Curr.Microbiol.App.Sci*. 9(02): 24-30.  
doi: <https://doi.org/10.20546/ijcmas.2020.902.005>