Research Article

Salmon Gonadotropin-Releasing Hormone Analogue (sGnRHa) as a Potent Ovulation Inducer for Artificial Insemination in Rabbit

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Abstract | This study evaluated the fertility of non-lactating rabbit does artificially induced with Salmon Gonadotropin-releasing hormone analogue (sGnRHa). Forty multiparous rabbit does were randomly divided into four groups of ten does each in a Completely Randomized Design (CRD). The does in the control were naturally mated; the does in the other groups were at the moment of Artificial Insemination (AI) intramuscularly injected with 5µg/kgBW, 10µ/kg/BW and 15µg/kgBW of sGnRHa in Ovaprim®, respectively. Blood samples were collected pre injection and at 60 and 120 minutes post insemination. Data recorded were Plasma LH levels, kindling rate, total kit born, total born alive, still born, weight of kits at birth and gestation period (days) and were analyzed using Analysis of variance of the General Linear Model procedure and t-test where appropriate. The plasma LH for the AI groups peaked at 60mins while it peaked at 120mins for the control. Kindling rates were 10%, 10%, 60% and 50% respectively for the groups. There was no significant difference ($p>0.05$, t-test) between the litter size and total live kits obtained for 10µg and 15µg groups. Kit weight at birth showed no statistical difference ($p>0.05$, ANOVA) among all the groups. There was no still born in the control and the 5µg group. The gestation period (days) for the control and 5µg group were 32.00±0.00 while the gestation period (days) between the 10µg and 15µg groups was not statistically different ($p>0.05$, t-test). It was concluded that sGnRHa could be a potent substance for inducing ovulation in low sexually receptive does submitted for AI.

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Introduction

Ovulation induction is necessary for artificial insemination (AI) in the rabbit doe due to the lack of stimuli normally evoked by the male at copulation. In rabbits, ovulation is a neuroendocrine reflex that is physiologically induced at the condition of natural mating (Schneider et al., 2006). Ovulation can be induced by the sight of a sexually active male, mating with a vasectomized male or a female, mechanical stimulation of the doe's vagina, visual, auditory and olfactory contact of the male (Laborda et al., 2008; Ola and Oyegbade, 2008; Kalaba and Abdul-Khalek, 2011). Artificially, ovulation can also be induced by intramuscular injection of either gonadotropin- releasing hormone (GnRH) or its synthetic analogues (Moce et al., 2003; Rommers et al., 2004) and intravaginally by the inclusion of GnRH and their analogues in the seminal dose (Taponen et al., 2003).

Artificial GnRH analogue (GnRHa) are produced when different amino acids are inserted at position 6 and 10, which are 50 to 100 times as potent (superactive) and more durable than the natural form (Fiçicioğlu et al., 2005). Ovaprim is a synthetic hormone preparation containing salmon gonadotropin releasing hormone analogue and domperidone (sGnRHa + Domperidone) which are usually used for spawning induction in catfishes to get quality fish seed (Sahoo et al., 2005). The potency may be attributed to high pituitary binding affinity and gonadotropin hormone releasing capacity.

Preliminary studies at the Obafemi Awolowo University Teaching and Research Farm had earlier shown that the cheaper and more readily available Ovaprim (sGnRHa) could also be a possible ovulation inducer for rabbits does. There is therefore a need to determine the optimum dosage that will achieve high productivity per doe. This will ultimately help to achieve sustainable rabbit production at a reduced cost. Thus, the objectives of this study were to establish the optimum dosage of sGnRHa for ovulation induction in rabbit does, determine the plasma LH pattern after intramuscular injection and evaluate the fertility performance of rabbit artificially induced with sGnRHa as compared to natural mating.

Materials and Methods

The study was conducted at a commercial rabbit farm within Ile-Ife, Nigeria. A total of 40 multiparous (1-3 parity) rabbit does (weight between 2.00-2.50kg) and matured fertile bucks (20.68-29.42kg) of crosses of New Zealand White, English Spot and Chinchilla were used mixed in the four groups. Each animal (doe and buck) was weighed using Electronic weighing balance (Citizen) MP-5000 Min: 20g: Max: 5000g. Animals were housed individually in cubicles (196×74×41) cm in row cages and each was provided with feed and water in cement pots *ad libitum* using rabbit pelletized ration containing 2700kcal/DE/kg and 17% crude protein (CP). The pens were covered with chicken wire mesh at the sides and planks at both ends and roofed with asbestos sheets. Prior to the commencement of the study, the pens were cleaned thoroughly and cages were disinfected with IZAL before the commencement of this study. Left over feed and water were removed and cages were cleaned daily. Feed and water pots were washed regularly and mange occurrence was promptly treated.

Experimental animals, design and treatments

Animals were randomly divided into 4 treatments containing 10 animals each in a completely randomized design (CRD). The treatments also involved 5 fertile bucks. Animals in the first treatment were naturally mated in a ratio of two does to a buck and this served as the control. The animals in groups 2-4 were injected intramuscularly with sGnRHa at 5µg/kg (T5), 10µg/kg (T10) and 15µg/kg (T15) of body weight, respectively and artificially inseminated thereafter. The sGnRHa was supplied from Ovaprim® which contains 20µg/ml of sGnRHa and 10mg of domperidone.

Semen used for the AI was obtained from the 5 fertile bucks; which was collected using an improvised artificial vaginal (Ola, 2016) and had been pre-examined under the microscope for motility, concentration, morphologically normal cells and the pH. The ejaculates were pooled, and diluted with Tris-Citrate glucose extender. Only ejaculates with a gel-free volume higher than 0.2ml and sperm motility (microscopic evaluation) higher than 70% were used. Does were vaginally inseminated with a pool of extended fresh semen using a disposable plastic pipette, receiving a dose of at least 20 million spermatozoa in a volume of 0.5ml.

Collection of blood samples and hormonal analysis

Five does were randomly chosen from each treatment and blood samples were collected from the ear vein into sample tubes immediately prior to natural mating or manual semen insemination and at 60 and 120 minutes after mating and AI to determine the plasma LH level. The samples were immediately centrifuged at 3000rpm for 15minutes and the plasma stored at -20°C until assay. The rabbit LH was analysed using Enzyme Immunoassay procedure (EIA) using rabbit luteinizing hormone LH ELISA Kit (EK151209C) from Biotain Pharma CO., Limited, Fujian, China. Conversion of the substrate by the enzyme was measured in an automatic plate reader (BIO-TEK EL×808 Ultra Microplate Reader, EL×808IU) immediately at 450 nm.

Data collection and statistical analysis

Data were collected on the pre- mating and AI weight of the does, Kindling rates, litter size, gestation length (days), total number of kits (live and dead) and live kit weight were recorded. On day 27 after AI and mating, nest boxes were placed in the cages and within 12

hours after kindling of each litter, the live kits weight were taken.

Data obtained in this study were statistically analyzed using One-Way Anova of the General Linear model of SPSS 17.0 software (SPSS Inc. Chicago, Illinois, USA). The difference between means was tested by Duncan's Multiple Range Test and T-test, where appropriate and differences considered significant at the $p<0.05$.

Results and Discussion

Effect of sGnRHa on plasma luteinizing hormone concentration in rabbit does

Figure 1 below showed the effect of varying dosages of sGnRHa on plasma lutenizing hormone concentration. There was no significant difference in the mean plasma concentration of LH at 0 min between the treatments while the mean concentrations of LH at 60 and 120 minutes for all the treatments differed significantly at $p<0.05$. The basal LH concentration in the treatment ranged from 6.86 to 7.27ng/ml, LH concentrations peaked in blood samples collected 60 minutes after AI (9.93±1.45 ng/ml ,11.50 ± 3.73 and 10.70 ± 3.10 ng/ml), respectively for T5, T10 and T15. In the naturally mated group, peak LH was detected at 120 minutes (7.73 ± 1.12 ng/ml). At 120 minutes after AI, the does in T10 and T15 had a basal LH concentration of 9.60 ± 0.77 and 9.53 ± 1.72 ng/ml, respectively. In the non-injected group LH concentration remained similar between the sampled periods (6.86±2.39ng/ml, 6.53±3.8ng/ml and 7.73±1.12ng/ml).

Results from this study showed that the efficiency of sGnRHa varied according to different dosages resulting in LH response in the does. In rabbit does, the peak LH concentrations have been found to occur 60-90 minutes after exogenous administration of GnRH and its analogue (Quintela et al., 2004; Kalaba and Abdel-Khalek, 2011; Rebollar et al., 2012; Zhang and Yinghe, 2012), this was confirmed in this study. However, the peak values of LH concentration obtained in this study were not in agreement with those obtained by Robellar et al. (2012) with intramuscular administration of Buserelin (276.3ng/ml) and Quintela et al. (2004) (35.1µg/ml). This may be due to the breed of rabbit used, the active ingredient in Ovaprim, the environmental effect and physiological state of the does used. The values of the LH concentration are affected by the sexual receptivity of does. Rodriguez et al. (1989) reported that LH concentration depend on sexual receptivity of does with the level increasing after 15 minutes of GnRH challenge depending on sexual receptivity of does and GnRH dose. Low sexual receptivity are associated with increased prolactin secretion which are associated with reduction in the number of binding receptors to LH in the follicle cells (Rodriquez et al., 1989) with a consequent reduction on the level of LH secretion at the moment of mating and AI.

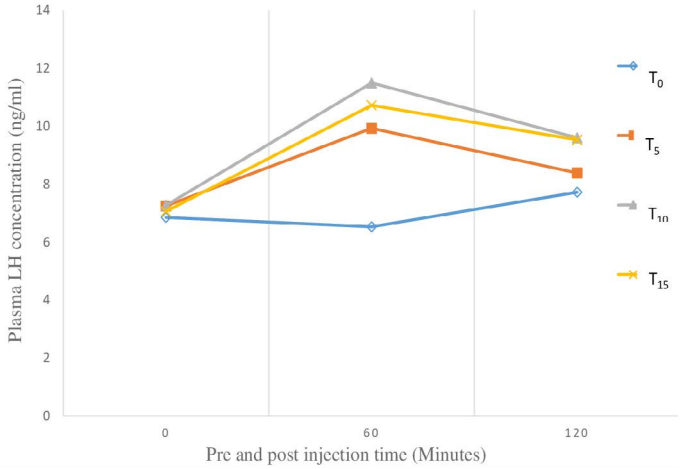

Figure 1: *Plasma LH concentrations before (0 mins) and after mating and AI (60 mins and 120 mins). T0: Naturally mated, T5:5µg, T10: 10 µg and T15: 15µg/ doe of sGnRHa.*

Pre mating and AI weight of does and the effects of administering varying levels of sGnRHa on the reproductive performance of does

The results in Table 1 showed the mean body weight of does before mating and AI and the reproductive performance of does administered varying dosages of sGnRHa. There was no significant difference ($p>0.05$) in the mean weight of the does before mating and AI.

The table also revealed that the group artificially induced by 10 µg/kg BW of sGnRHa (T10) had the highest kindling rates of 60% followed by 15 µg/kg BW of sGnRHa (T15) with 50% while does in groups T0 and T5 (naturally mated and 5 µg/kg BW of sGnRHa) had 10 % each. The result obtained for the litter size and total live kits revealed that there was no significant variation ($p>0.05$) for the mean of the T10 and T15 treatments using t- test. The highest litter size value of 5.67±1.86 was obtained for the does in T10 while 4.50 ±3.51 was obtained for T15 while T0 and T5 the lowest mean values of 3.00±0.00 and 2.00±0.00, respectively. The total live kits were not significantly ($p>0.05$) different between

Table 1: *Mean ± SD of pre- mating and AI weight of does and reproductive performance of does administered varying dosages of sGnRHa*

	sGnRHa Dosages				
Parameters	T_0	T_5	T_{10}	T_{15}	**P values**
Weight of Doe (Kg)	2137.40 ± 259.05	2369.40 ± 178.01	2242.60 ± 298.48	2282.20 ± 435.46	0.131
Kindling Rate (%)	10	10	60	50	-
Litter Size	3.00 ± 0.00	2.00 ± 0.00	5.67 ± 1.86	4.50 ± 2.17	0.068
Total Live Kit	3.00±0.00	2.00±0.00	5.20±2.17	4.40±3.51	0.058
Still Born	0.00±0.00	0.00±0.00	1.00±0.00	1.33±0.00	-
Gestation Period (days)	32.00 ± 0.00	32.00 ± 0.00	30.33 ± 0.55	30.65 ± 0.52	0.70
Kit Weight at Birth(Kg)	44.00 ± 6.25	46.50 ± 3.54	37.88 ± 8.15	38.56 ± 3.67	0.087

Level of significance (p>0.05, Duncan Multiple Range Test and t-test); T0: naturally mated; T5: 5µg/kg sGnRHa; T10: 10µg/kg sGnRHa; T15: 15µg/kg sGnRHa.

the means of T10 and T15 (5.20±2.17 vs. 4.40±2.65). T0 had mean live kits of 3.00±0.00 while the lowest value of 2.00 ± 0.00 was obtained for T5. There was no significant difference (p>0.05) for the weight of all kits between the treatments. However, the control showed numerically higher kit weight at birth (46.50 ± 3.54g) as compared to 44.00± 6.25g, 37.88±8.15g and 38.56±3.67g for the induced group, respectively. The T15 had higher still born value of 1.33±0.00 over T10 (1.00±0.00). T0 and T5 had no still born. The gestation length of the naturally mated group (T0) and T5 was 32.00±0.00 days while the mean gestation period of the T10 and T15 were lower 30.33±0.55 and 30.65±0.52 but were not significantly different (p>0.05, t-test) from each other

Live weight of does had been found to have a significant effect on reproductive performance. El-Magawry et al. (1988), Kumar et al. (2005) and Mahmoud (2013) reported a significant effect of dam body weight on litter size while Lebas et al. (1986), submitted that ovulation potential which affects prolificacy increases on the average with the does size. The litter size at birth, the number of total litter born alive and litter birth weight in this study in relation to doe body weight is comparable to what was obtained by Orunmuyi et al. (2006) and Fayeye and Ayorinde (2008). These authors concluded that majority of the rabbit stock in Nigeria have undergone some decline in breed purity over the years and there may be the need to totally replace them with new imports or improve them through selection and crossbreeding.

The kindling rates of 60% obtained for the T10 was similar to 62.74% obtained by Ondruska et al. (2008) when 2.5 µg of (GnRH) lecirelinum was administered intramuscularly after AI and also in agreement with the 60 % kindling rate obtained by Ewuola et al. (2015), when buserelin was administered intramuscularly at a dose of 0.8µg, 1.2µg and 1.6µg. The 50% kindling rates in T15 Confirmed the findings of Rodriquez and Ubilla (1998) who observed that higher doses only partially increased the ovulation rate. The 5µg was not sufficient to cause ovulation in the majority of the doe resulting in low kindling rates. Improvement in kindling rates of rabbit does treated with 10µg and 15µg doses of sGnRHa over the naturally mated group in this study may be due to the effect of GnRH on the ovary which includes the stimulation of oocyte maturation, follicular development, corpus luteum formation (Zanagnolo et al., 1996). GnRH induces oocyte maturation through activation of specific GnRH receptors on granulosa cells (Koves et al., 1989). Similarly, GnRH stimulates prostaglandin synthesis in pre-ovulatory follicles and increasing concentration of prostaglandin plays an important role in oocyte maturation (Calder et al., 2001).

The value of litter size obtained for the T0 in this study is not in agreement with the 4.08±1.3 for New Zealand White and 5.75±0.9 for Chinchilla obtained by Oke and Iheanocho (2011) when the two breeds were naturally mated. The low litter size obtained in the natural mated group may be due to low sexual receptivity of the does and stress associated with force mating. Gonzalez- Mariscal et al. (2007) reported that low sexual receptivity negatively impacts reproductive performance of does with only sexually receptive does becoming pregnant. Low sexual response is associated with an increase in prolactin, which has been related to a reduction in the number of binding receptors to LH in the follicle cells which determines

the imminent ovulation rate as well as the number of breakage of pre-ovulatory follicles thus determining the number of implanted embryos and litter size.

Rashwan et al. (1995) reported that differences in litter size at birth could be due to differences in ovulation rate, and pre-implantation viability as well as maternal effects and it is determined by the number of matured, fertilized and established ova. Higher incidence of stillbirth was recorded with the use of higher dose of sGnRHa confirming the findings of Zapletal and Pavlik (2008) who found out higher incidence of abortion and stillbirth while using higher level of lecirelin.

Though, there was no significant difference in kit body weight ($p>0.05$) across the treatments, it has however been reported (Kalaba and Abdul-Khalek, 2011; Mahmoud, 2013), that increased number of kit per litter is associated with the decreased birth weight, the number of fetuses in the uterus or the uterine horn, intrauterine localization; and maternal nutrient distribution during pregnancy.

Shorter gestation period in T10 and T15 groups is in tandem with the findings of Hilmy (1999) and Abdel Azeem et al. (2012) who reported that gestation period decreases linearly with increase in litter size. This may be due to milk production and growth of fetuses which represent a great burden to the doe (Rashwan et al., 2003).

Conclusions and Recommendations

This study evidently revealed that sGnRHa could be a potent substance for inducing ovulation in rabbit does, through intramuscular injection with the dosage of 10µ/kg/BW giving the best result. However, further studies will be necessary to determine the effect of the domperidone contained in Ovaprim in lactating does.

Authors Contributions

The publication is part of Mr. Bamidele Olufisayo's Master's degree thesis supervised by Prof. Ola Safiriyu Idowu. The research idea was conceived, the study supervised and manuscript proof read by Ola Safiriyu Idowu while Bamidele Olufisayo designed, carried out the study and prepared the manuscript for publication.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article

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