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Ovulation Induction in Rabbit Does by Intramuscular Administration of Salmon Gonadotropin-Releasing Hormone Analogue (sGnRHa)

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Abstracts

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/kg BW, 10 µg/kg BW and 15 µg/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 kits born, total born alive, stillborn, 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 60 mins 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 stillborn in the control and the 5µg group. The gestation periods for the control and 5µg group were 32.00 ± 0.00 while the gestation periods (days) for the 10µg and 15µg groups were 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 rabbit does.

Keywords: Rabbit, Gonadotropin-Releasing Hormone, Ovulation, Artificial Insemination

Introduction

Ovulation induction is a required step for an Artificial Insemination protocol in the rabbit does due to the lack of stimuli normally evoked by the male at copulation. Artificially, ovulation can be induced in does by intramuscular injection of either gonadotropin-releasing hormone (GnRH) or its synthetic analogues (Rommers *et al.*, 2004) and intravaginally by the inclusion of GnRH and their analogues in the seminal dose (Quintela *et al.*, 2004).

Ovaprim is a synthetic hormone preparation containing salmon gonadotropin-releasing hormone analogue and domperidone (sGnRHa + Domperidone) normally used for spawning induction in catfishes (Sahoo *et al.*, 2005).

Preliminary studies at our research farm had earlier shown that the cheaper and more readily available Ovaprim (sGnRHa) could also be a possible ovulation inducer for rabbit 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

A total of 40 multiparous (1-3 parity) rabbit does (weighing 2.00-2.50 kg) and 5 mature fertile bucks (20.68-29.42 kg) of crosses of New Zealand White, English Spot and Chinchilla were used. The animals were allotted equally into 4 groups 1-4. Groups 2-4 were injected intramuscularly with sGnRHa at 5µg/kg (T_2), 10 µg/kg (T_3) and 15 µg/kg (T_4) 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 insemination was obtained from 5 fertile bucks using an improvised artificial vagina (Ola, 2016). Ejaculate samples from the 5 bucks were pre-examined under the microscope for motility, concentration, morphologically normal cells and pH. On-the-field ejaculates were pooled, and diluted with Tris-Citrate glucose extender. Only ejaculates with a gel-free volume higher than 0.2ml and sperm motility (field 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. Does in group 1 (T_1) were naturally mated (readily and forced mating) at pairing rate of 1 buck to 2 does. On day 27 after insemination or mating, nest boxes were placed in the cages and within 12 hours after kindling of each litter, the live kits were weighed. 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. Blood samples were collected from the ear veins of 5 randomly selected does from each group prior to and after mating and AI to determine the LH level. The samples were centrifuged at 3000rpm for 15 mins and plasma stored at -20°C until assay.

Data obtained 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 differences were considered significant at the $p < 0.05$.

Results and Discussion

The effect of varying doses of sGnRHa on plasma luteinizing hormone concentration showed there was no significant difference in the mean plasma concentration of LH at the point of sGnRH injection. The plasma LH concentrations peaked in blood samples collected 60 minutes after injection. In the naturally mated group, the peak LH was detected at 120mins after mating, even then the value was below those recorded for the injected groups.

Figure 1: Plasma LH concentrations before (0 mins) and after mating and AI (60mins and 120mins). T_0 : Naturally mated, T_2 : 5 μg , T_3 : 10 μg and T_4 : 15 μg / doe of sGnRHa.

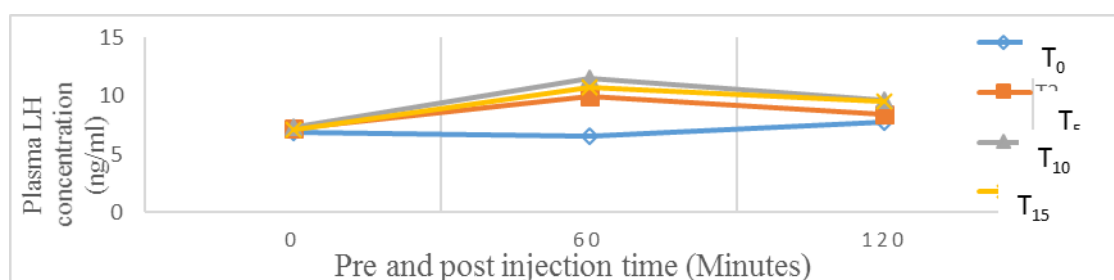


Table 1 revealed that T_{10} had the highest kindling rate of 60% followed by T_{15} with 50% while does in groups T_0 and T_2 had 10 % each. The gestation length T_0 and T_2 was 32.00 ± 0.00 days while the mean gestation period of the T_3 and T_4 were lower (30.33 ± 0.55 and 30.65 ± 0.52 but were not significantly different ($p > 0.05$, t-test).

Table1: Mean $\pm$ SD of Pre-mating/Inseminated Weight of Does and Reproductive Performance of Does Administered Varying Dosages of sGnRHa

Parameters	sGnRHa Dosages				P Level
	T_0	T_2	T_3	T_4	
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), T_0 = naturally mated, T_2 = 5 $\mu\text{g}/\text{kg}$ sGnRHa, T_3 = 10 $\mu\text{g}/\text{kg}$ sGnRHa, T_4 = 15 $\mu\text{g}/\text{kg}$ sGnRHa

The litter size at birth, the number of kits 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). Dam body size has been found to significantly affect the prolificacy, with ovulation potential increasing with dam body size (Mahmoud, 2013). 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; Robellar *et al.* 2012). The present study aligned with this assertion. The peak values obtained in this study were lower than those obtained by Robellar *et al.* (2012) with intramuscular administration of Buserelin (276.3ng/ml) and Quintela *et al.*, 2004 with 0.8 µg Buserelin (35.1µg/ml). This may be due to the breed, the active ingredient in Ovaprim, the environmental effect and physiological state of the does used. Rodriguez and Ubilla (1989) reported that LH concentrations depend on sexual receptivity of does. The kindling rates of 60% obtained for the T₃ was similar to 62.74% obtained by Ondruska *et al.* (2008) when 2.5 µg of (GnRH) lecorelin 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 doses of 0.8 µg, 1.2 µg and 1.6 µg, respectively. The 50% kindling rates in T₄ confirmed the findings of Rodriguez and Ubilla (1989) who observed that higher doses only partially increased the ovulation rate. The low litter size obtained in the naturally mated group may be due to low sexual receptivity of the does and stress associated with forced mating.

Low sexual receptivity negatively impacted reproductive performance of does with only sexually receptive does becoming pregnant (Castellini *et al.*, 2010). 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. The higher total live kits in T₃ and T₄ as compared to the stillbirth may be due to the multiparity of the does used. The number of live kit and fertility had been found to be high in multiparous than nulliparous and primiparous does (Perrier *et al.*, 1998). 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, as observed in this study, it has however been reported (Kalaba and Abdul-Khalek, 2011), that increased number of kits per litter is associated with 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 T₃ and T₄ groups is in tandem with the findings of Abdel Azeem *et al.* (2012) who reported that gestation period decreased linearly with increase in litter size. This might be due to milk production and growth of fetuses which represented a great burden to the doe (Rashwan *et al.*, 2003).

Conclusion and Recommendation

It was concluded that sGnRHa could be a potent substance for inducing ovulation in rabbit does, through intramuscular injection. However further studies will be necessary to determine the effect of the domperidone contained in Ovaprim in lactating does.

References

- Abdel-Azeem A.S., H.A. Abdel-Azim, and M. S. Ragab M.S. (2012). Effects of prostaglandinF2 Alpha injection and rabbit dosage on reproductive, hematological parameters and level of progesterone. *Egypt Poultry Science*, 32: 189-199.
- Ondruska, I, V. Parkanyi, and I. Chlebee.(2008). Effect of LHRH analogue induced in seminal dose on kindling rate and prolificacy of rabbits artificially inseminated. *In: Proceeding of the 9th World Rabbit Congress, Verona, Italy*, Pp 423-433.
- Ewuola, E., O. Ojeniyi., A. Adeyemi., A. Lawson., and A. Oni.(2015). Effect of Buserelin Acetate on the fertility rate of synchronized rabbit does artificially inseminated. *Tropentag*, Sept., 16-18, 2015, Berlin, Germany
- Fayeye.T.R, and K. L. Ayorinde.(2008.) Effects of season, generation, number of mating, parity and doe number of teat on doe and litter birth characteristics in domestic rabbit *9th World Rabbit Congress – June 10-13, Verona, Italy*
- Kalaba Z.M., and A.E. Abdel-Khalek. (2011). Effect of repeated GnRH injection and intra-vaginal Mechanical stimulation on reproductive performance of doe rabbits. *Asian Journal of Animal and Veterinary Advances*, 6, 1010-1018.
- Ola, S.I. (2016). OLIRAV: A simple disposable rabbit artificial vagina device/procedure. *Proceedings of the 11th World Rabbit Conference*, 15-18 June 2016, Qingdao, China, pg 209-212.
- Orunmuyi M., I. A. Adeyinka, O. A. Ojo, and F. D. Adeyinka. (2006). Genetic parameter estimates for pre-weaning litter traits in rabbits. *Volume 9, Issue 15. Directory of open access Journals*.
- Perrier, G, M., Theau-Clément, B. Poujardieu, and G. Delhomme. (1998). Essai de conservation de

- lasemence de lapin pendant 72 heures. In *Proc.: 7èmes Journées de la RechercheCunicole*, 13-14 Mai, Lyon, France, 237-240.
- Quintela. L.A, A.I. Pena, M.D. Vega, J. Gullon and M.C. Prieto. (2004). Ovulation induction in rabbitdoes submitted to artificial insemination by adding buserelin to the seminal dose. *Journal of Reproduction and Nutritional. Development*. 44: 78-88.
- Rashwan A.A., Z. Szendro, Z. Matics, A. Szalai,E. Biro-Nemeth, and I. Nagy.(2003). Effects of the time of insemination and litter size on the gestation length of rabbits. *World rabbit Science*, 1175-83
- Robellar, P.G,A. Dal Bosco, P. Millán, R. Cardinali, G. Brecchia, L. Sylla, P.L. Lorenzo, andC.Castellini. (2012). Ovulating induction methods in rabbit does: The pituitary and ovarian responses *Theriogenology*Volume 77, Issue 2, 15 January 2012, Pages 292–298
- Rodriquez J.M, and E. Ubilla.(1998). Effects of sexual receptivity on ovulation response in rabbitsdoesinduced with GnRH. *Proceeding. 4th World Rabbit Congress, Budapest*, 504-509
- Rommers, J.M., R. Meijerhof, J.P. Noordhuizen and B. Kemp, (2004). The effect of level offeeding. Inearly gestation on reproductive success in young rabbit does. *Animal. Reproduction Science*. 81:151-158
- Sahoo. S.K., S. S. Giri, and A. K. Sahu. (2005). Induced spawning of Asian catfish, *Clariasbatrachus*(Linn): effects of various latency periods and sGnRH α and domperidone dose on spawning performance and egg quality. *Acquacultural Resources* 36:1273.
- Zapletal, D;and A. Pavlik. (2008). The effect of lecirelin (GnRH) dosage on the reproductiveperformance of nulliparous and lactating rabbit does. *Animal Reproduction Science*, 104: 306-315