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RELATIONSHIP BETWEEN ENDOGENOUS ESTRADIOL LEVELS AND ABILITY TO EXOGENOUS GONADOTROPIN RELEASING HORMONE IN RABBITS.

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ABSTRACT

Responses to ovulation in rabbits depend on neural reflexes after natural mating. The stage of follicular growth and levels of estradiol (E_2) could also be involved. Injection of gonadotropin releasing hormone (GnRH) gives rise to ovulation similar to natural mating 10 White New Zealand does were allocated to two groups. Control group in which two blood samples was taken for E_2 determination before Iml GnRH intra-muscular injection for ovulation induction. Second group was considered to be non-receptive for natural mating and treated with GnRH.
In conclusion, levels of E_2 were positively correlated to ovulation induction ability in control group. Whereas ovulation induction by mean of GnRH failed to demonstrate ovulation in second group.
Keywords : Rabbits – Doe – Ovulation – GnRH - Gonadotropin

INTRODUCTION

Ovulation induction in rabbits is very necessary for Artificial Insemination (A.I) application. Ovulation process can be synchronized by a number of different hormonal regimes basing on synthetic gonadotropin releasing hormone (Espey, 1982; Fan *et al* ., 1988; Bonanno *et al* ., 1990; Bourdillon *et al* ., 1992).

Doe receptivity through checking color of the vulva is the major sign to predict its ability to ovulation (Maertens and Luzi, 1995). Forced mating and blind A.I can result low fertility percent and low litter size at born. The aim of this work is to find out relationship between E_2 levels prior to GnRH injection and doe ability to ovulate for both receptive and non-receptive does.

MATERIALS AND METHODS

Doe condition& receptivity:

Ten White New Zealand (WNZ) does were allocated into two groups according to their mating ability. Does weight ranged from 2.400 to 3.650 Kg with an average of 3.100 Kg. While the does age averaged 2 years old. Does were kept at a rabbitry in Experimental Farm of Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Lighting system was 16 hr light / 8 hr dark. Does were subjected to ad libitum feeding (17% crude protein, 12% crude fiber, 2% fat and 2600 Kcal DE/kg feed) and free access of water via metal nipples provided in the doe metal cages. Does were chosen to be in the same physiological condition as regards pups weaning. Signs of doe receptivity to mating is characterized by vagina color, calmness to capture and handling and appearance of external reproductive organs as regards its

turgidity due to high blood supply. Does which do not represent receptivity signs were considered to be non-receptive.

Blood samples collection& E_2 determination:

All does were subjected to blood sample collection. Where two blood samples were taken from intermediate ear vein 2 hours before ovulation induction occurrence. Blood samples were subjected to centrifugation 3000 rpm for 15 min to separate blood serum which kept at – 20 C° until use. One sample was taken for each hour before ovulation induction to determine E_2 profile by means of Radio Immunoassay (RIA) technique (COAT. A. COUNT. Estradiol, DPC, Diagnostic Products Corporation, Los Angeles, CA 90045-5597, Cat./ Lot No: TKE 211752).

Ovulation induction method & Artificial Insemination A.I technique:

Two groups of does were treated with 1 ml GnRH (inducel GnRH, Gonadorelina 2 mg, Excipiente 100 ml, Laboratorios Ovejero, S.A., Peregrinos, s/n. Leon, Espana) intra-muscular IM injection for ovulation induction method. For receptive does group (control group) 1 ml GnRH was used and for second group of non-receptive does. For Artificial Insemination A.I technique, semen samples with 70 % advanced motility was pooled and diluted 1 : 3 with Tris buffer- free of egg yolk extender. For A.I procedure, does were hold in the up side down position for A.I. Where 1 ml of extended semen was used to provide more than 50 million sperm for each insemination. After 10 days of A.I, does were palpated for conception rate estimate. After parturition, litter size traits were recorded as well.

Statistical analysis:

Data as regards E_2 levels were analyzed for two groups by using ANOVA test of SPSS program. Also correlation between E_2 profile and conception rate and litter size traits was estimated for two groups.

RESULTS AND DISCUSSION

Levels of oestradiol before ovulation induction:

Data of 17 ß-oestradiol (E_2) are presented in Table 1& Figure 1. There were differences between two groups as regards E_2 levels but these differences were not significant ($P < 0.05$) for both to estimates of E_2 profile for two successive hours before ovulation induction method. Control group has a higher values of E_2 than second, especially at second hour before ovulation induction method. In which average levels of E_2 for two successive hours were 24.05 ±8.36 and 16.73 ±3.64 for first (control) and second group, as shown in Figure 1. These results could explain the ability of control group for ovulation. Many authors (Kermabon, *et al* ., 1994; Rebollar, *et al* ., 1992; Ubilla and Rebollar, 1995) mentioned that, a receptive doe generally exhibits a higher number of large follicles and a higher oestrogen level with respect to non-receptive ones. Also change % of E_2 levels of two groups can detect degree of E_2 persistence for secretion during 2 hours before ovulation

induction especially for first group (control). In which summation of change % for first and second hour was 48 and 32% for first and second group, as shown in Table 1. Where higher oestrogen levels could be responsible for doe receptivity as was detected for control group (Table 2).In addition oestrogen secretion is correlated with waves of ovarian follicles growth and stage. Where control of folliculogenesis lies with the gonadotropins and local regulatory factors in the ovary such as steroids, cytokines and growth factors (Findlay *et al* ., 1998). Local E_2 levels near to the ovarian blood vessels could produce main effects of E_2 prior to and during oestrous phase of the doe. These high levels of local E_2 levels can affect peripheral E_2 levels of blood circulation as obtained in this experiment.

Effect of gonadotropin releasing hormone(GnRH) injection

Gonadotropin releasing hormone (GnRH) is involved in the cascade initiation of reproductive events which leads to ovulation process. There is a particular emphasis on the roles of local regulators in the acquisition and modulation of responsiveness of ovarian cells to gonadotropins, and their roles in proliferation and differentiation (Findlay *et. al.*, 1996). While in this experiment, these events did not occur as shown in Table 2 as regards second group. This is may be attributed to the lake of E_2 levels for second group which is necessary for the initiation of positive feedback mechanism between ovaries (E_2- hormone) and anterior pituitary (gonadotropins) especially LH to achieve ovulation process. In addition, failing in ovulation for the second group could be attributed to the insufficient dose of injected GnRH to initiate required stimulation for ovulation induction. On the other hand, a combinations of follicle stimulating hormone (FSH) or pregnant mares serum gonadotropin (PMSG), followed by the administration of either human chorionic gonadotropin (hcg), luteinising hormone (LH) or gonadotropin releasing hormone (GnRH) sufficiently initiates induction of ovulation (Besenfelder and Brem, 2000).

Number of ovulated does, conception rate and Litter size traits:

In this experiment 3 from 10 does were ovulated and conceived for two groups by the application of A.I. Where 3 does were ovulated and belonged to the first group of 5 receptive does. In which, ovulated does and conception rate can account 60 % of total does of first group. This is in agreement with Cecchini *et al* ., (1992). Where they reported that, the overall conception rate on the 40 farms was 78 % ranging from a minimum of 50 % in one case to over 85 %. Mean of litter size traits of first group is presented in Table 2. Correlation coefficient between litter size born and mean level of E_2 for two successive hours before ovulation induction was $r = 0.38$ and was not significant for two groups. This can prove the participation of E_2 –levels in ovulation process and prove also that there is some other factors beside estrogen that can regulate this phenomena of ovulation and conception. Also variation in the conception % have been observed within the same farm due to season or disease or to unknown factors (Zanirato, 1988).

In general, it could be concluded that, it is very necessary to examine estrous manifestation for each doe before natural mating or Artificial

Insemination application instead of applying forced mating or blind AI. The doe ability for ovulation by using different hormonal regimes depends on endogenous estradiol levels of doe prior to natural mating or AI program. It is important from practical point of view to collect a blood sample from doe which do not show a clear manifestation of estrous to determine its ability for ovulation.

Table 1. Means of E_2 levels ±S.E of doe blood serum for two successive hours before GnRH injection for ovulation induction method for two groups.

Estradiol (Pg/ml)	First group*(g)	Second g.**	Mean ±S.E
First hour	22.1±9.9 [a]	12.5±4.2 [a]	16.3±7.1 [A]
Change % of Mean	+35%	-23%	0%
Second hour	26.0±5.7 [a]	21.0±5.5 [a]	23.0±3.5 [A]
Change % of Mean	+13%	-9%	0%
Sum of Change %	48%	32%	0%

*** First group: Receptive does and injected with 1ml of GnRH for ovulation induction**
**** Second group: Non-receptive does and injected with 1ml of GnRH for ovulation induction.**

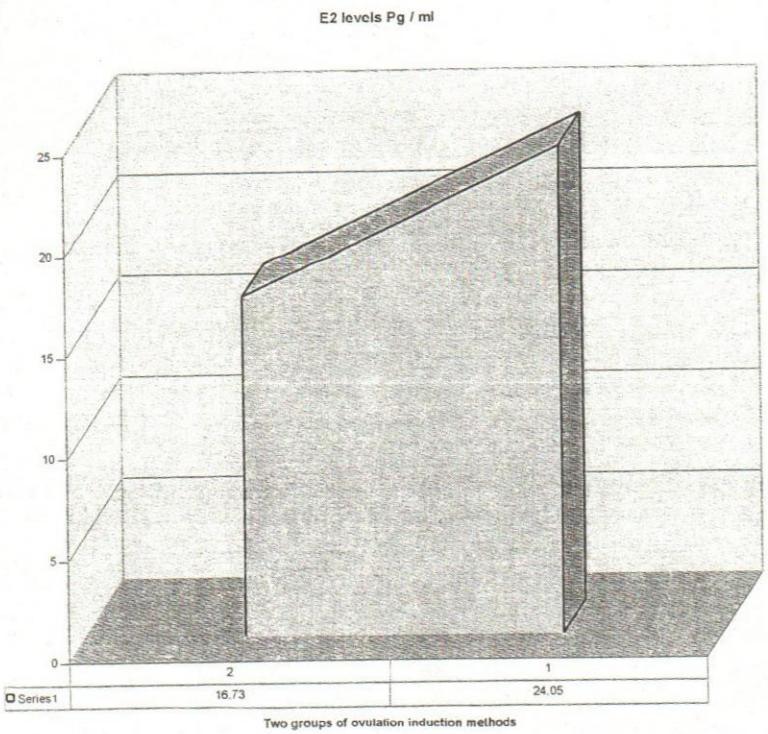

Fig. 1. Average of E_2 levels for two successive hours for two groups(1 and 2) which do not differ significantly.

Table 2. Mean of ovulated does for two groups of ovulation induction method and Litter size traits.

Treatment	No of ovulated does / 5	Mean ±S.E of litter size traits			
		LS[1]	LSA[2]	LSD[3]	LSW[4]
First group*	3/5	6.33 ±0.88	5.67 ±0.33	0.67 ±0.67	480g ±20.82
Second g.**	0/5	0	0	0	0

LS[1] : Litter size born
LSA[2] : Litter size born alive
LSD[3] : Litter size born dead
LSW[4] : Litter size weight (gram)

* First group: Receptive does and injected with 1ml of GnRH for ovulation induction

** Second group: Non-receptive does and injected with 1ml of GnRH for ovulation induction.

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العلاقة بين مستوى هرمون الإستراديول والقابلية للحقن بالهرمون المنبهة لإفراز الهرمونات الجونادوترفية فى إناث الأرانب

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الاستجابة للتبويض فى إناث الأرانب تعتمد على التنبيه العصبى الناتج عن عملية التلقيح الطبيعى. وكذلك فأن درجة نمو وتطور الحويصلات المبيضية ومستوى إفراز هرمون الإستراديول لهما تأثير كبير على عملية التبويض. أيضا فأن الحقن بالهرمون المنبهة لإفراز الهرمونات الجونادوترفية يؤدى إلى حدوث التبويض بشكل مشابهة للتبويض الناتج عن عملية التلقيح الطبيعى. لذلك فأنة قد تم تقسيم ١٠ من إناث الأرانب النيوزلندى الأبيض إلى مجموعتين. المجموعة الأولى وهى مجموعة المقارنة وفيها تم أخذ عينتين من الدم لتقدير مستوى تركيز هرمون الإستراديول وذلك قبل حقن ١ ملى من الهرمون المنبهة لإفراز الهرمونات الجونادوترفية فى العضل وذلك لاستحداث التبويض. أما المجموعة الثانية فاعتبرت غير قابلة للتلقيح وتم معاملتها بالهرمون المنبهة لإفراز الهرمونات الجونادوترفية. وبصفة عامة فقد وجد ارتباط بين مستوى هرمون الإستراديول والقابلية للتبويض وذلك فى مجموعة المقارنة. أما استحداث التبويض باستخدام الحقن بالهرمون المنبهة لإفراز الهرمونات الجونادوترفية فلم تتمكن من استحداث التبويض فى المجموعة الثانية .