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Effects of a single administration of different gonadotropins on day 7 post-insemination on pregnancy outcomes of rabbit does

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ABSTRACT

This study aimed to investigate the effects of a single administration of one of three different gonadotropins on Day 7 post-insemination on ovarian activity, progesterone (P_4) concentration and pregnancy outcomes of rabbit does. Multiparous, non-lactating, V-line does were artificially inseminated after synchronization and ovulation induction with equine chorionic gonadotropin (eCG; 25 IU im) and gonadotropin releasing hormone (GnRH; 0.8 μg buserelin im) 48 h later. On Day 7 post-inseminarion, does were randomly allocated into four groups (n = 40/group). Does of each group were intramuscularly injected with a single dose of one of physiological saline (*placebo*; control), GnRH (0.8 μg buserelin), human chorionic gonadotropin (hCG; 25 IU) or eCG (25 IU). Concentration of serum P_4 was determined on Days 6, 9, 11 and 18 post-insemination. On Day 14 post-insemination, the ovaries and reproductive tracts of pregnant does were removed and weighed. Also, numbers of visible follicles, hemorrhagic follicles, corpora lutea of pregnancy (pCLs), new CLs (nCLs; formed after Day 7 post-insemination) and implantation sites were recorded. Conception rate, parturition rate, abortion rate, litter size/weight and litter viability were recorded. The highest ($P < 0.05$) reproductive tract and ovary weights were for eCG. The highest ($P < 0.05$) number of visible ovarian follicles was for eCG, whereas the lowest ($P < 0.05$) was for GnRH. Treatment with eCG increased ($P < 0.05$) numbers of pCLs and total implantation sites compared to the other groups. Treatment with GnRH or hCG increased ($P < 0.05$) number of nCLs compared to control and eCG. The highest rate of fetal loss was in does treated with GnRH. The concentration of serum P_4 decreased ($P < 0.05$) following the treatment with GnRH and continued low until Day 18. However, it remained in line for control, hCG and eCG groups up to Day 11, then decreased ($P < 0.05$) for control and hCG on Day 18, being lower for hCG than control, while continued to increase for eCG up to Day 18. Compared to control, treatment with eCG improved ($P < 0.05$) conception and parturition rates by 24 and 22%; respectively, while GnRH and hCG treatments decreased ($P < 0.05$) them by 57 and 47.6%; respectively. Litter size and litter weight at birth were improved by eCG, but were adversely affectd by GnRH and hCG. In conclusion, a single administration of eCG 7 Days post-insemination could be recommended for improving pregnancy outcomes in rabbits.

1. Introduction

An approach to achieve a successful reproductive management regim in rabbit (*Oryctolagus cuniculus*) farms is to improve pregnancy outcomes by minimizing total and/or partial early embryonic loss. In most mammals, up to 60% of all pregnancies are terminated at the end of the per-implantation period [1]. In rabbits, the majority of fetal losses (66%) occurs between days 8–17 of gestation [2]. Pre-implantation period represents a critical stage for embryo survival and placental establishment [3], because it is contaminant with the beginning of embryo–maternal interface [1]. Success of implantation and thus maintenance of pregnancy is a complex process that is equally contributed by both maternal and embryonic sides [4].

During pre-implantation period, early developing embryos synthesize different molecules such as hCG in human, eCG in equines, estradiol (E_2) in pigs, interferon τ in cattle and gonadotropin-like substance in rabbit [5], signaling their presence to the dam [4]. Gonadotropins released by an embryo have an endocrine action via rescue of the corpus luteum (CL) from luteolysis and thus improving

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subsequent P_4 release during pregnancy. Also, they have paracrine actions on the endometrium affecting decidualization, angiogenesis, immune-modulation and matrix remodeling, preparing for embryonic implantation [6].

Several studies demonstrated that, in some animal species rather rabbits, a single administration of exogenous gonadotropins such as hCG [7], GnRH [8] or eCG [9] around the pre-implantation period has been found to improve pregnancy outcomes via different mechanisms, including adequancy in luteal P_4 synthesis and enhancements in placental characteristics. In rabbits, luteal P_4 is required during the whole period of pregnancy as the doe rabbit is luteal P_4 dependent for maintaining pregnancy [10]. Inadequacy of P_4 level in pregnant doe causes insufficient embryonic development which may lead to termination of pregnancy [11]. Earlier *(in vitro)* studies performed on rabbits have reported that P_4 synthesis from the luteal tissue could be evoked in response to either luteinizing hormone (LH) or hCG [12–14]. However, to our knowledge, the effect of gonadotropins administration around implantation time on rabbit reproductive performance has not been studied. On the base of previous facts, it was hypothesized that administration of GnRH, hCG or eCG around implantation time may bring about positive effects on luteal P_4 synthesis and, consequently, on reproductive performance of rabbit does. Therefore, the effects of a single administration of GnRH, hCG or eCG on day 7 post-insemination, representing first day of implantation in rabbits, on ovarian activity, P_4 concentration, embryo implantation and pregnancy outcomes of rabbit does were investigated.

2. Materials and methods

2.1. Animals and experimental design

The present study was carried out at the Laboratory of Rabbit Physiology Research, Agricultural Experimental Station, Faculty of Agriculture, Alexandria University, Alexandria (31° 20′N, 30° E), Egypt. Multiparous, non-lactating, V-line rabbit does (n = 120; 3.17 ± 0.15 kg body weigh) handled according to the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes were used. Does were individually housed in standard galvanized wire cage batteries (40 × 50 × 35 cm) in naturally ventilated and lighted rabbitry (10.97 ± 0.36 h daylight length, 23.73 ± 0.1 °C temperature and 71.50 ± 0.33% relative humidity). Animals were fed on pelletted diet (18.90% CP and 10.25 MJ/kg DE), covering their daily nutritional requirements according to National Research Council [15]. Clean tap water was offered excessively.

Estrus synchronization was accomplished by treating each doe with equine chorionic gonadotropin (eCG; 25 IU im; Gonaser®, Hipra, Spain); 48 h later gonadotropin releasing hormone (GnRH; 0.8 μg buserelin im; Receptal, Boxmeer, Holland) was administered to induce ovulation and does were immediately artificially inseminated with 0.2 mL (15×10^6 sperm/insimination) of fresh diluted (1:5) pooled semen collected from previously proven-fertile rabbit bucks. On day 7 post-insemination, does were randomly allocated into four experimental groups (n = 40/group). Does of each group were injected im with single dose of one of physiological saline (*placebo*, control), GnRH (0.8 μg buserelin im), human chorionic gonadotropin (hCG; 25 IU im; Epifasi, Eipico, Egypt) or eCG (25 IU im). The experimental plan is depicted in Fig. 1.

2.2. Determination of progesterone

Blood samples were collected from the ear vein of each doe into non-heparinized tubes in the morning of Days 6, 9, 11 and 18 post-insemination. Serum was obtained from blood samples by centrifugation at 700×*g* for 20 min and then stored at −20 °C. Concentration of P_4 was determined in serum samples of does confirmed pregnant (n = 7/group) to avoid variation in serum P_4 patterns due to pseudopregnancy and not related to the treatment [16]. Concentrations of serum P_4 were measured using solid-phase enzyme immunoassay commercial kit (Monobind Inc., USA). The lower limit of assay detection was 0.10 ng/mL and the intra- and inter-assay CVs were 10.3 and 11.6%, respectively.

2.3. Examination of ovarian structure and reproductive tract

On Day 14 post-insemination, six does/group confirmed to be pregnant by abdominal palpation on Day 10 post-insemination were euthanized [17]. The ovaries and reproductive tract of each doe were dissecte and weighted after removing adhered fat masses and connective tissues. Ovarian structures, including visible follicles, hemorrhagic follicles, corpora lutea of pregnancy (pCLs, corpora lutea formed due to ovulation of the ovulatory follicles at the time of ovulation induction and insemination) and new CLs (nCLs formed after Day 7 post-insemination due to the gonadotropins administration) were counted. Total implantation sites along each uterine horn were counted and classified according to presence or absence (decidual reactions or atrophic placentas without fetus) of fetus [18] (Fig. 2). Each implantation site was dissected and weighed (weight of fetal vesicle).

2.4. Evaluation of fertility and pregnancy outcomes

Fertility parameters of does were estimated as: conception rate, % = no. of pregnant does on Day 10/total no. of inseminated does × 100; parturition rate, % = no. of delivering does/no. of inseminated does × 100 and abortion rate, % = no. of does aborted/no. of pregnant does × 100. Pregnancy outcomes parameters were estimated as litter size at birth or weaning = no. of kids at birth or weaning/no. of kindling does and as viability rate at birth or weaning = no. of live kids at birth or weaning/litter size at birth or weaning.

2.5. Statistical analysis

The fixed effects of treatments (control, GnRH, hCG and eCG) on

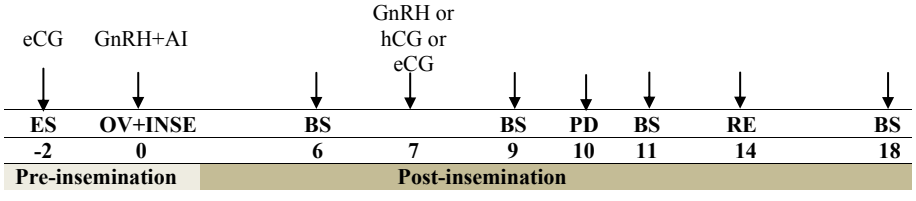

Fig. 1. Schematic frame work of the study (**ES** = estrus synchronization, **eCG** = equine chorionic gonadotropin, **GnRH** = gonadotropin releasing hormone, **AI** = artificial insemination (Day 0), **OV** + **INSE** = ovulation induction and insemination, **hCG** = human chorionic gonadotropin, **Bs** = blood sampling, **PD** = pregnancy diagnosis and **RE** = reproductive tract examination).