



Adverse effects of repeated doses of Ivermectin alone or with the combination of vitamin C on reproductive system of female rabbits

KEYWORDS

Ivermectin, Vitamin C, Hormone, Fertility, Female Rabbits.

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ABSTRACT

This study was designed to investigate the effect of repeated administration of Ivermectin alone or with combination of vitamin C on sexual hormones. Besides, an attempt had been done to demonstrate the effect of ivermectin on fertility and pregnancy efficacy of female rabbits. Ninety six mature female rabbits were used in this study and two experiments had been done. Each experiment were divided into 8 groups of equal number (6) as follows: group one was administered 0.9% NaCl which act as control, Group two, three & four were administered (0.5mg/Kg, 1mg/Kg, 2mg/Kg B.W Ivermectin) respectively, Group five was administered 50mg/Kg B.W vitamin C only, Group six, seven & eight were administered 50mg/Kg vitamin C in addition to Ivermectin (0.5mg, 1mg, 2mg respectively). The Ivermectin was given S/C weekly, while vitamin C was given daily P.O. for 8 weeks.

The results showed significant decrease ($P < 0.05$) in Estradiol level in 3rd, 4th, 6th, 7th & 8th groups, while progesterone level showed significant increase ($P < 0.05$) in 5th & 7th groups, the FSH level postulated significant increase ($P < 0.05$) in 4th, 5th & 6th groups, meanwhile, the LH level did not show significant difference in all treated groups as compared with the control group. The results of fertility study revealed adverse effect of ivermectin therapy on fertility and block the pregnancy in all treated group except the fifth group which administered vitamin C only as compared with control group and their effect persistent in second mating in intermediate dose (1mg/Kg Ivermectin) and high dose (2mg/Kg Ivermectin) groups alone and with combination with vitamin C. In conclusion: Ivermectin has adverse effects on reproductive efficacy on female rabbits, as well as, the vitamin C has not well emphasized beneficial effects on reproduction.

Introduction

Ivermectin is a semisynthetic macrocyclic lactone and acts as a broad spectrum antiparasitic drug against many internal and external parasites (1). It consists of a mixture of two compounds 22,23 dihydro-22,23-dihydro-2,2,6,6-tetramethyl-5,8-dioxabicyclo[2,2,1]heptan-2-one (H2B1a) and 22,23 dihydro-22,23-dihydro-2,2,6,6-tetramethyl-5,8-dioxabicyclo[2,2,1]heptan-2-one (H2B1b). Moreover, it contains no less than 80% H2B1a and no more than 20% H2B1b (2). Other studies have been used vitamin A and E in combination of ivermectin (3). The literature survey showed that ivermectin determined effect on the reproductive performance of ewe during the breeding season (4). However, many studies showed an adverse effect of ivermectin on the male reproductive system such as on goat (5), bull (6), ram (7). Little research has been published on the adverse effect of ivermectin on female reproduction, as well as, up to our knowledge no previous studies had been done on ivermectin with vitamin C combination, so it was deemed of interest to investigate the effect of repeated administration of ivermectin on the reproductive system of female rabbits and also to highlight whether the combination of vitamin C with ivermectin has an ameliorative effect.

Materials and Methods

The Ivermectin 10% was purchased from the local market (VET Product Office, KIPRO Company, Holland) and Vitamin C 500mg (Al-Shahba Labo, Syria). The FSH, LH & progesterone were done according to appropriate Elisa kit from Human, GmbH Ltd. Germany, while Estradiol from Monobind Inc. Lake Forest, CA 92630, USA.

Animal housing

Ninety six mature female rabbits (*Lepus cuniculus*), (1200-2000gm) body weight and (8-12 months) of age were brought from the local market in Basra Province/ Iraq. The rabbits were housed (6 rabbits / cage) in wire mesh cages measuring (100 X 50 X 50 cm) under controlled animal house conditions at temperature (25 ± 3 °C) and relative humidity (50 ± 5 %) in the animal house of Veterinary Medicine College in Basra University. The rabbits were kept under observation for one month. The animals were offered *ad libitum* a rabbit's diet, green leaves, alfalfa, and water.

Experimental Design

Two experiments had been done:

I-Hormonal Experiment

Forty eight mature female rabbits were divided into eight groups (6 rabbits in each group). Each group was treated for 8 weeks as follows: **Group one:** Injected (0.9 % NaCl) which acts as a control, **Group two:** Injected (0.5 mg/kg B.W Ivermectin), **Group three:** Injected (1mg/kg B.W Ivermectin), **Group four:** Injected (2 mg/kg B.W Ivermectin), **Group five:** administered 50mg/ Kg B.W Vitamin C only, **Group six:** Injected (0.5 mg/Kg B.W Ivermectin + 50mg/kg B.W Vit.C), **Group seven:** Injected (1mg/kg B.W Ivermectin + 50mg/Kg B.W Vit. C), and **Group eight:** Injected (2mg/ Kg B.W Ivermectin + 50mg/kg B.W Vit. C). The Ivermectin was given subcutaneously and weekly, while vitamin C was given daily and orally. At the end of the experiment (8 Weeks), the blood samples were taken directly from the heart by using

disposable syringe and put in screw tube without anticoagulant then centrifuged at 4000 rpm for 10 minutes to get serum for hormonal assay (E2, Proges. FSH&LH).

II-Fertility Experiment

Another Forty-eight mature female rabbits were similarly chosen randomly and divided into 8 groups of equal number (6 rabbit/ group) and the treatment were also similar to the first experiments. After 8 week of treatment, a known fertile male rabbits (2 male/group) were mated with the female rabbits in each groups to estimate the pregnancy and number of offspring's after one month, then the mating was extended to another one months to study persistent the effect of treatment.

Statistical analysis

The results were analysed by one- way ANOVA test. When significant differences were found, the means were compared using least significant difference (LSD). All statistical calculations were carried out by the aid of the statistical SPSS V. 22 (SPSS Inc.).

Results

Experiment one

Hormonal assay

The effect of Ivermectin alone or with the combination of vitamin C on sexual hormones (Estradiol, progesterone, Follicular stimulating hormone and luteinizing

hormone) of female rabbits after 8 weeks of treatment clarified in Table (1).The Estradiol (E2) level in the 3rd, 4th, 6th, 7th, and 8th groups revealed significant decrease ($p < 0.05$) in Estradiol level as compared with control group. The progesterone level showed significant increase ($p < 0.05$) in 5th and 7th.The follicular stimulating hormone (FSH)in 4th, 5th and 6th groups clarified significant increase ($p < 0.05$)in level in comparison with control group. However, the luteinizing hormone (LH) in all treated groups showed no significant differences as compared with control group.

Experiment Two Fertility and pregnancy

As illustrated on Table (2), after 8 weeks of treatment, in first mating there are absent of pregnancy in all treated groups except in control group which administered normal saline and group which given 50mg/Kg B.W vitamin C daily.

At second mating there are notice pregnancy in second and sixth groups which injected 0.5mg/Kg B.W Ivermectin alone and group which injected 0.5mg/Kg B.W Ivermectin+ vitamin C, in addition to control group and group five which administered vitamin C only.

Table (1): Effect of ivermectin alone or with the combination of vitamin C on sexual hormone in female rabbits after 8 weeks of treatment. (Mean \pm SE), n=6/ group

	E2 ng/ml	Prog. ng/ml	FSH ng/ml	LH ng/ml
1 st Group Control 0.9%Nacl	133.166 \pm 95.477 a	2.976 \pm 0.885 cd	4.250 \pm 0.384 b	1.433 \pm 0.790
2 nd Group (0.5mg/Kg IVM)	102.083 \pm 62.837 abc	3.566 \pm 0.786 bcd	7.000 \pm 0.563 bc	1.150 \pm 0.882
3 rd Group (1mg/Kg IVM)	7.500 \pm 7.500 c	4.450 \pm 1.482 bcd	6.833 \pm 1.181 bc	0.416 \pm 0.238
4 th Group (2mg/Kg IVM)	6.083 \pm 6.083 bc	8.350 \pm 3.484 ac	8.066 \pm 0.623 ac	1.400 \pm 1.129
5 th Group 50mg/Kg Vit.C	27.500 \pm 17.689 ac	8.975 \pm 2.658 ab	11.033 \pm 1.723 ac	0.900 \pm 0.601
6 th Group (0.5mg/Kg IVM) +(50mg/Kg Vit.C)	9.166 \pm 9.166 bc	2.433 \pm 0.518 d	10.991 \pm 1.198 ae	1.866 \pm 0.945
7 th Group (1mg/Kg IVM) + (50mg/Kg Vit.C)	11.000 \pm 7.724 bc	10.725 \pm 2.541 a	6.200 \pm 0.879 bc	0.300 \pm 0.136
8 th Group (2mg/Kg IVM) + (50mg/Kg Vit.C)	9.333 \pm 9.333 bc	3.400 \pm 1.264 bcd	6.175 \pm 1.315 bc	1.900 \pm 1.191

* Estradiol (E2) , progesterone(proge), follicle stimulating hormone(FSH), luteinizing hormone(LH), Vitamin (Vit.).

*The different letters denote significant differences ($p < 0.05$) between groups..

Table (2): Effect of Ivermectin alone and with combination of vitamin C on fertility and pregnancy efficacy of female rabbits after 8 weeks of treatment. n=6/ group.

	First mating		Second mating	
	NO. pregnant	NO. offspring	No. pregnant	NO. offspring
1 st Group Control 0.9% Nacl	5	16	5	16
2 nd Group 0.5mg/Kg IVM	0	0	1	3
3 rd Group 1mg/Kg IVM	0	0	0	0
4 th Group 2mg/Kg IVM	0	0	0	0
5 th Group 50mg/Kg Vit.C	6	20	6	20
6 th Group 0.5mg/Kg IVM + 50mg/Kg Vit.C	0	0	2	6
7 th Group 1mg/Kg IVM + 50mg/Kg Vit.C	0	0	0	0
8 th Group 2mg/Kg IVM + 50mg/Kg Vit.C	0	0	0	0

Discussion

Generally, the results of present study revealed significant decrease in Estradiol in all groups which administered ivermectin alone and groups which received ivermectin in combination with vitamin C except the (2nd and 5th group). While progesterone showed significant increase in group which administered 50mg/Kg of vitamin C (group 5th) and group which given 1mg/kg Ivermectin plus vitamin C (group 7). The FSH revealed significant increase in groups which given high dose of Ivermectin(2mg), and 5th group (vitamin C only) as well as, 6th group which given 0.5mg/Kg Ivermectin plus vitamin C.

Its well-known that ivermectin is lipophilic compound, for that reason it prefers to accumulate in fatty tissue which is an important reservoir of the drug and this can influence persistent in the body and its pharmacokinetics behaviour. The lipophilic character of ivermectin also accelerate the distribution extensively from blood stream to different tissues (8,9).

The endocrine disruptor resulted in this study due to excess production of free radicals which form by repeat administration of ivermectin and it is very clear that ivermectin attend to accumulate in fatty tissues particularly ovaries.

In normal case the secretion of LH, FSH is occur under the control of hypothalamus- pituitary action (10). Some researchers found that any environmental compound antagonizing or mimicking steroid hormone action could presumably change the glycosylation of LH and FSH by reducing their biological activity (11). Normally ovaries could produce increasing concentrations of progesterone in response to LH surge, as well as, vitamin C act to stimulate its production (12). Richards, (13) clarified that estrogen effect on granulosa cell acts to amplify overall action of FSH. The increasing of progesterone will lead to differentiating granulosa cell through FSH induction of cytochrome P450 sc (CYP11A) which act to catalyse the rate limited conversion of cholesterol to pregnenolone (14).

This finding is in agreement with (15) who noted the ingestion of 2.13mg/animal/day of abamectin caused significant increase in follicular stimulate hormone (FSH) in male rats. In another study found the subcutaneous injection of Ivermectin (0.15 mg, 0.2mg /Kg) caused increase in progesterone level in camel during the breeding season, while the animal which treated outside the season showed decrease in progesterone level (16). However, in endocrine study by (17) about the effect of ivermectin in heifers, they noted no change in estradiol, FSH level during the experiment, while the mean LH level were decreased in untreated heifer 4 weeks before the first estrus and the amplitude of LH pulses was increased in treated heifers when reach to puberty. On the other hand, some investigators (18) emphasized the continuous treatment of ivermectin in dairy heifers caused increasing the progesterone level. Vitamin C acts as enhancer for gonadotropins secretion by enhancement the secretion of endogenous norepinephrine (19). Similar finding postulated the Abamectin caused an elevation in FSH level, while LH level did not change significantly in male rats (20)

In present study in first mating, the ivermectin therapy cause block to pregnancy in all treated groups except group 5th which administered vitamin C only as compared with control group. While in second mating, the ivermectin effect will be resolution in 2nd group which administered (0.5mg/Kg IVM) alone and with combination with vitamin C

(group 6th) and the animals become pregnant.

The result of our study is disagreement with (21) who concluded the dose of 0.6mg/Kg of Ivermectin which given monthly for 8 treatment could not cause adverse effect on spermatogenesis, fertility and reproductive performance on beagle dogs. The oral administration of 0.4mg/Kg /day of Ivermectin caused toxic effect on neonate rats which was related not to in utero exposure, but it refers to postnatal exposure through maternal and this toxicity occur due to postnatal formation of the blood brain barrier in rats (22). This results occur may be due to the alteration in hormones were caused endocrine disruptor as a results of free radical production and this lead to reproductive toxicity. Some researcher postulated the free radicals can results in oxidative stress which lead to sperm or ovum damage, deformity, endometriosis, preeclampsia, miscarriage, intrauterine growth retardation and infertility (23,24). Reactive oxygen species could be generated directly from oocytes and embryo or from their surrounding which in turn lead to mediate the processes of embryonic development (25). The reactive oxygen species could causes multiple physiological process from oocyte maturation to fertilization, embryo development and pregnancy (23). Some researchers noted the free radicals exert an important roles in reproductive system especially for ovulation (26).

Furthermore, some researcher postulated the therapeutic dose of Ivermectin (0.2mg/Kg) did not interfere or impair the sexual behaviour of inexperienced male rats, while the use of 1mg/Kg of Ivermectin caused an impairment the appetitive phase and previous sexual experience blocked almost entirely this sexual impairment and they suggested that previous sexual exerts a positive effect in attenuating the sexual impairment produced by Ivermectin treatment (27).

In conclusion: Ivermectin has adverse effects on reproductive efficacy on female rabbits, as well as, the vitamin C has not well emphasized beneficial effects on reproduction.

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