The biochemical effects of ivermectin on reproductive hormones and mineral homeostasis in Baladi cows post parturition

Kadry M. Sadek[1]*, and Hazem M. Shaheen[2]

[1]Department of Biochemistry, Faculty of Veterinary Medicine, Damanhur University, Egypt

[2]Department of Pharmacology, Faculty of Veterinary Medicine, Damanhur University, Egypt

---

SADEK, K. M., H. M. SHAHEEN: The biochemical effects of ivermectin on reproductive hormones and mineral homeostasis in Baladi cows post parturition. Vet. arhiv 85, 95-103, 2015.

ABSTRACT

This study investigated the effect of an antiparasitic ivermectin (IVM) drug on the endocrine reproductive hormones of twenty healthy adult 250-350 kg Baladi cows that were 4-7 years of age. The cows were divided into two groups (n = 10 each). The first was a control group injected with physiological saline; the second group was treated with the recommended therapeutic dose of IVM (0.2 mg/kg, s/c) one day after parturition. Blood samples were taken on the 1st, 15th, 30th and 90th day after IVM treatment. The results revealed that IVM injection at one day after parturition delayed estrous for up to 3 months (absence of estrous signs and rectal palpation revealed no ovarian structures). IVM significantly (P<0.05) decreased serum follicular stimulating hormone (FSH), luteinizing hormone (LH) and estradiol for up to 3 months. In addition, IVM significantly (P<0.05) increased serum progesterone, prolactin and cortisol for up to 3 months. Further, IVM caused unobservable changes in serum testosterone and sex hormones binding globulin. Serum calcium levels significantly increased on the 1st day of IVM injection, while serum phosphorus significantly decreased on the 15th and 30th day of IVM injection. It was concluded that IVM delayed estrous in cows for three months via disturbances in the female reproductive hormones and calcium/phosphorus homeostasis. Therefore, it is recommended that IVM should not be injected directly after parturition. Furthermore, the increased calcium after IVM indicates that an overdose of IVM should not be counteracted by calcium therapy; instead, any other antiallergic preparation could be used.

Key words: cows, ivermectin, mineral homeostasis, post parturition, reproductive hormones

Introduction

Ivermectin (IVM) belongs to a family of compounds (avermectins, which also include abamectin, emamectin, eprinomectin and doramectin) that are produced by the

---

*Corresponding author:

Kadry M. Sadek, PhD., Department of Biochemistry, Faculty of Veterinary Medicine, Damanhur University, Egypt, Phone: +0100 3695 012; (+2) 045 3591 018; Fax: (+2) 045 3591 018; E-mail: ksaadek@yahoo.com

microorganism actinomycete *Streptomyces avermitilis*, which was first isolated from soil in Japan (PARADIS, 1986). The structure of IVM is similar to that of macrolide antibiotics, but it appears to lack any antibacterial or antifungal activities. IVM was introduced to the market in 1981 as a veterinary antiparasitic drug and soon proved to be the most effective broad spectrum antiparasitic drug ever developed (WOODWARD, 2011). Five years after its introduction, IVM was registered for use in 46 countries and was being used worldwide to treat approximately 320 million cattle, 151 million sheep, 21 million horses and 5.7 million pigs. In simple-stomached animals, 95 % of oral IVM is absorbed (PLUMB, 2002). Its absorption after oral dosing is more rapid than that after subcutaneous administration, although there is greater bioavailability after subcutaneous administration. IVM concentrates in the liver and body fat (BEASLEY, 1990). It is excreted in the feces and has a low degree of liver metabolism. The persistence of the parent compound in the body may partly explain the long half-life, which is reported to be 2 to 3 days. Due to its rapid and specific antiparasitic and anthelmintic action, IVM was proposed to be an agonist for neurotransmitter function. Experimental studies confirmed this hypothesis by demonstrating that inhibition occurred via glutamate-gated chloride ion channels and invertebrate-specific glutamate-gated anion channels in peripheral neuromuscular synapses, thus suppressing nerve impulse conduction (ÕMURA, 2002). IVM interacts with these channels, thereby preventing their closure. Consequently, synapse membranes become increasingly permeable to chloride ions, which leads to the hyperpolarization of the neuronal membrane, and decreases or prevents neuronal transmission. This, in turn, leads to paralysis of the somatic muscles, particularly the pharyngeal pump, causing the death of the parasite (ARBONA et al., 2010). The γ-aminobutyric acid (GABA)-related chloride ion channels, which are present only in nematodes, insects and ticks, are only inhibited with greater drug concentrations (TURNER and SCHAEFFER, 1989). The same authors revealed that, in mammals, GABA receptors and neurons are found in the central nervous system, whereas in arthropods and nematodes, they are located in the peripheral nervous system.

The location of GABA receptors, coupled with the relatively low doses needed, ensures that mammals can ingest IVM with a high degree of safety. IVM is effectively and widely used against numerous endoparasites and ectoparasites, especially nematodes and arthropods (CHACCOUR et al., 2013). However, IVM exhibits a broad spectrum of activity against gastrointestinal and lung nematodes and against ectoparasites of clinical relevance in domestic animals (SUÁREZ et al., 2013). Two conflicting and inconsistent viewpoints about the effects of IVM on animal reproduction are prevalent among scientists. Namely, some researchers feel that IVM has dangerous and hazardous effects on reproduction (COUSENS et al., 1997). Others feel that IVM can have beneficial effects on reproductive efficiency in animals (LANKAS et al., 1989). Due to these conflicting views, this study aimed to evaluate the possible deleterious effects of IVM on reproduction and

to determine whether these effects were temporary or permanent, neither of which has been determined previously.

Materials and methods

Twenty mature Baladi cows, aged 4-7 years and weighing 250-350 kg, were used in this study. This study was performed from 15 July 2013 to 15 October 2013. The cows were divided randomly into 2 groups, with each group containing 10 animals. The first group was a control group and was injected with physiological saline; the second was treated with the recommended therapeutic dose of IVM (1 % w/v solution of IVM, V.M.D. Ltd, Arendonk, Belgium, 0.2 mg/kg, s/c) one day after parturition. Blood samples of 10 mL were collected via jugular venipuncture on the 1st, 15th, 30th and 90th days post injection. Sera were harvested via centrifugation at 3,000 rpm for 15 minutes and stored at -20 °C until biochemical analysis. Sera were used for the assessment of testosterone, estradiol, FSH, LH, prolactin, progesterone, sex hormones binding globulin (SHBG), cortisol, calcium and phosphorus. All biochemical parameters were analyzed using commercially available kit methods. Unico 2100 UV-Spectrophotometers, ELx800 Absorbance Microplate Reader and other laboratory equipment aids were used for biochemical analysis. Moreover, each parameter was performed according to the instructions of its kit.

Statistical analysis. The Student's *t*-test (independent sample test) was used to compare the treated and control groups. The data were expressed as the means ± standard error of means (SEM). The differences were considered statistically significant at P<0.05.

Results

General signs. The clinical examination revealed absence of estrous signs and rectal palpation revealed no ovarian structures up to three months post IVM injection.

Serum testosterone and estradiol. Compared to the control group, the serum testosterone level in the IVM-treated group was not affected throughout the experiment. However, serum estradiol significantly decreased (P<0.05) in concentration by the 15th and 30th days and returned to the control value by the 90th day (Table 1).

Table 1. Effects of IVM on serum testosterone and estradiol in Baladi cows post parturition

Groups		Serum testosterone (ng/mL)	Serum estradiol (pg/mL)
Control		0.82 ± 0.02[a]	139 ± 4.66[a]
Treated	1st day	0.76 ± 0.04[a]	135 ± 5.45[ab]
	15th day	0.87 ± 0.01[a]	87 ± 3.13[c]
	30th day	0.81 ± 0.02[a]	63 ± 2.89[d]
	90th day	0.85 ± 0.06[a]	142 ± 5.73[a]

Means within the same column carrying different letters are significantly different (P<0.05)

Serum FSH and LH. Compared with the control animals, the serum FSH level significantly decreased (P<0.05; Table 2) by the 15th and 30th days in the IVM-treated cows. The serum LH level significantly decreased by the 30th day. Both hormones returned to the control level by the 90th day.

Table 2. Effects of IVM on serum FSH and LH in Baladi cows post parturition

Groups		Serum FSH (mIU/mL)	Serum LH (mIU/mL)
Control		8.9 ± 1.35[a]	16.4 ± 2.65[b]
Treated	1st day	9.1 ± 0.87[a]	18.1 ± 3.09[a]
	15th day	7.2 ± 1.41[b]	16.7 ± 2.76[b]
	30th day	7.4 ± 1.12[b]	13.2 ± 1.77[c]
	90th day	8.8 ± 0.91[a]	16.2 ± 3.17[b]

Means within the same column carrying different letters are significantly different (P<0.05).

Serum prolactin and progesterone. Table 3 shows that cows in the IVM-treated group had a significant increase in serum prolactin and serum progesterone on the 1st, 15th, and 30th days compared with the placebo group (P<0.05). The level of progesterone hormone returned to normal levels on the 90th day, while the prolactin level was still significantly increased (P<0.05) compared with control group.

Table 3. Effects of IVM on serum prolactin and progesterone in Baladi cows post parturition

Groups		Serum prolactin (ng/mL)	Serum progesterone (ng/mL)
Control		16.1 ± 2.04[c]	0.9 ± 0.05[c]
Treated	1st day	21.7 ± 3.23[b]	1.7 ± 0.83[b]
	15th day	22.3 ± 2.66[b]	3.2 ± 0.58[a]
	30th day	29.7 ± 3.18[a]	1.9 ± 0.11[b]
	90th day	21.8 ± 1.85[b]	1.1 ± 0.09[c]

Means within the same column carrying different letters are significantly different (P<0.05)

Table 4. Effects of IVM on serum SHBG and cortisol in Baladi cows post parturition

Groups		SHBG (nmol/L)	Cortisol (µg/dL)
Control		69.1 ± 5.74[a]	7.89 ± 1.36[b]
Treated	1st day	68.4 ± 6.67[a]	13.61 ± 3.78[a]
	15th day	63.2 ± 4.18[b]	12.99 ± 1.00[a]
	30th day	69.3 ± 8.38[a]	14.47 ± 2.93[a]
	90th day	68.7 ± 5.89[a]	9.17 ± 1.62[b]

Means within the same column carrying different letters are significantly different (P<0.05)

Changes in serum cortisol level and sex hormones binding globulin. Table 4 shows that serum cortisol was significantly ($P<0.05$) increased on the 1st, 15th and 30th days post injection in the IVM-treated group, compared with the control group. However, the sex hormones binding globulin showed no changes throughout the experimental period.

Table 5. Effects of IVM on serum calcium and phosphorus in Baladi cows post parturition

Groups		Calcium (mg/dL)	Phosphorus (mg/dL)
Control		9.85 ± 0.89^b	5.75 ± 0.74^a
Treated	1st day	13.76 ± 1.66^a	5.29 ± 1.55^a
	15th day	10.33 ± 0.90^b	2.16 ± 0.05^b
	30th day	10.19 ± 1.22^b	2.86 ± 0.08^b
	90th day	9.25 ± 1.36^b	4.88 ± 0.68^a

Means within the same column carrying different letters are significantly different ($P<0.05$)

Serum calcium and phosphorus. Table 5 shows a significant increase in serum calcium levels on the 1st day post injection in the IVM-treated group, compared with the control group ($P<0.05$). The serum phosphorus were significantly ($P<0.05$) decreased on the 15th and 30th days following IVM treatment. On the 90th day, both calcium and phosphorus returned to the normal control values.

Discussion

The effects of IVM on selected reproductive hormones in Baladi cows were studied. The current findings in this study demonstrate that serum FSH, LH and estradiol levels undergo dramatic changes, as represented by a sharp decrease after IVM injection, but return to their normal values on the 90th day following the injection.

Further, IVM injection significantly increased serum progesterone, prolactin and cortisol levels, and these levels returned to control values by the 90th day post injection. Serum sex hormones binding globulin and testosterone were not affected throughout the experiment. IVM caused disturbances in calcium/phosphorus homeostasis, which was reflected in a significantly increased calcium level on the 1st day post IVM injection, and significantly decreased the phosphorus level on the 15th and 30th days post IVM injection. This effect is due to the high bioavailability of IVM and its long duration of action (TOUTAIN et al., 1997). The administration of IVM eventually caused significant pathological alterations that primarily affected the genital organs of female guinea pigs which may affect their reproductive performance (AL-HIZAB and HASSIEB, 2010). The effects of IVM on GABA-mediated neural transmission and the ability of GABA to suppress gonadotropin secretion combined with drug distribution and clearance (CHIU et al., 1990) subsequently suppressed normal ovarian function. In addition, IVM

decreased semen parameters and serum testosterone and follicle-stimulating hormone in Red Sokoto buck and serum testosterone in Iraqi Awassi ram, respectively (NAOMAN, 2012). Thus, IVM should be used cautiously in bucks and rams that are meant for breeding due to the deleterious effects observed on the animals' fertility parameters. There was an observable increase in serum progesterone level when IVM was administered during the breeding season in female camels and ewes, respectively (SERI et al., 2000). The reason for the increase in progesterone is not clearly known and may be considered to be either of ovarian or adrenal origin (BULMAN and LAMMING, 1978). The hypothalamo-hypophyseal negative feedback of progesterone could be responsible for decreased FSH and LH and, subsequently, decreased estrogen associated with delayed estrous after IVM injection, as observed in the current study. IVM is an irritant and subcutaneous drug that causes pain to the animal at the site of injection (BURG et al., 1979). This pain could explain the increased serum cortisol level. Nevertheless, MEJÍA et al. (1999) concluded that IVM treatment in dairy heifers may increase growth rate during development, advance the onset of ovarian function (earlier puberty) and positively affect the yearling pelvic area. The treatment of heifers at weaning with IVM increased the number of animals in estrous at the end of the feeding period (BAUCK et al., 1992). The same authors anticipated that any improvement in reproductive performance would occur strictly as a result of improvements in the body weight of heifers.

A number of studies have been performed investigating the involvement of insulin and the IGF family in IVM-induced ovarian activity (YELICH et al., 1996). These studies involved the regulation of neuroendocrine events in the hypothalamo-pituitary (MONGET and MARTIN, 1997) and at ovarian levels (CHASE et al., 1998). Antiparasitic treatment in heifers appears to be associated with early puberty onset and improved fertility (PURVIS and WHITTIER, 1996). However, continuous treatment with IVM from birth to puberty caused no differences in serum FSH, estradiol, or thyroxine levels between the treated and untreated heifers (LACAU-MENGIDO et al., 2000). IVM has no detrimental effect on the reproductive performance of ewes during the breeding season (KEISLES et al., 1993), which is reflected in the lack of alterations in the interval from PGF2ά-induced luteal regression to the onset of estrous between IVM- and control-treated ewes. WHITTIER et al. (1999) observed no LH variation in response to IVM treatment despite observing increased follicular development.

This discrepancy may be attributed to the species, age, seasonal variations of the ovarian activity or breed variation of animals used, and the dosage of drugs administered (PAUL and TRANQUILLI, 1986). Climatic variations may also be a factor responsible for these differences.

Conclusion

The results obtained in this study provide convincing evidence that strongly support producers' concern about the detrimental effect of IVM on fertility. IVM delayed estrous in cows for three months (absence of estrous signs and rectal palpation revealed no ovarian structures) via disturbances in female reproductive hormones and calcium/phosphorus homeostasis. Thus, IVM should not be injected directly after parturition. Furthermore, the increased calcium level observed post IVM injection indicates that an overdose of IVM should not be counteracted using calcium therapy. Any other antiallergic preparations should be applied instead. The mechanisms by which IVM treatment influences the female reproductive hormones need to be ascertained. We suggest that further studies are needed to clarify the relationship between IVM and the reproductive hormone profile at the molecular level.

References

AL-HIZAB, F. A., M. M. HASSIEB (2010): Histopathologic studies on the effect of repeated doses of Dectomax on some genital organs of female guinea pigs. Scientific J. King Faisal Univ. 11, 207-215.

ARBONA, R. J., N. S. LIPMAN, E. R. RIEDEL, F. R. WOLF (2010): Treatment and eradication of murine fur mites: Toxicologic evaluation of ivermectin-compounded feed. J. Am. Ass. Lab. Anim. Sci. 49, 564-570.

BAUCK, S. W., C. A. PICHE, K. M. NEWCOMB (1992): Effect of antiparasitic treatment in beef replacement heifers. Canadian Vet. J. 33, 394-396.

BEASLEY, V. R. (1990): A Systems Affected Approach to Veterinary Toxicology. Urbana, University of Illinois, pp. 181.

BULLMAN, D. E., G. E. LAMMING (1978): Milk progesterone levels in relation to conception, repeat breeding and factors influencing acyclicity in dairy cows. J. Reprod. Fert. 54, 447-458.

BURG, R. W., B. M. MILLER, E. E. BAKER, J. BIMBAUM, J. A. CURRIE, R. HARMAN, V. L. KONG, R. MONAGHAN, G. OLSON, I. PUTTER, J. P. TUNAC, H. WALLICK, E. O. STAPLEY, R. OIWA, S. OMURA (1979): The action of avermectin on identified central neurons from helix and its interaction with acetylcholine and gamma-aminobutyric acid response. Antimicrob. Agents Chemother. 15, 361-367.

CHACCOUR, C. J., K. C. KOBYLINSKI, Q. BASSAT, T. BOUSEMA, C. DRAKELEY (2013): Ivermectin to reduce malaria transmission: A research agenda for a promising new tool for elimination. Malaria J. 12, 153-161.

CHASE, C. C., A. C. KIRBY, T. A. HAMMOND, H. OLSON, M. C. LUCY (1998): Patterns of ovarian growth and development in cattle with a growth hormone receptor deficiency. J. Anim. Sci. 76, 212-219.

CHIU, S. L., M. L. GREEN, F. P. BAYLIS, D. ELINE, A. ROSEGAY, H. MERIWETHER, T. A. JACOB (1990): Absorption, tissue distribution, and excretion of tritium-labeled ivermectin in cattle, sheep, and rat. J. Agric. Food Chem. 38, 2072-2079.

COUSENS, S. N., A. CASSELS-BROWN, I. MURDOCH, O. E. BABALOLA, D. JATAU, N. D. ALEXANDER, J. E. EVANS, P. DANBOYI, A. ABIOSE, B. R. JONES (1997): Impact of annual dosing with Ivermectin on progression of onchocercal visual field loss. Bull. World Health Org. 75, 229-236.

KEISLES, D. H., C. M. V. BETTENCOURT, R. J. MOFFATT (1993): Effects of ivermectin on reproductive functions in ewes. J. Anim. Sci. 71, 2293-2296.

LACAU-MENGIDO, I. M., M. E. MEJÍA, G. S. DÍAZ-TORGA, I. A. GONZALEZ, N. FORMÍA, C. LIBERTUN, D. BECÚ-VILLALOBOS (2000): Endocrine studies in ivermectin-treated heifers from birth to puberty. J. Anim. Sci. 78, 817-824.

LANKAS, G. R., D. H. MINSKER, R. T. ROBERTSON (1989): Effects of Ivermectin on reproduction and neonatal toxicity in rats. Food and Chem. Toxicol. 27, 523-529.

MEJÍA, M., A. GONZALEZ-IGLESIAS, G. S. DÍAZ-TORGA, P. VILLAFAÑ, N. FORMÍA, C. LIBERTUN, D. BECÚ-VILLALOBOS, I. M. LACAU-MENGIDO (1999): Effects of Continuous Ivermectin Treatment from Birth to Puberty on Growth and Reproduction in Dairy Heifers. J. Anim. Sci. 77, 1329-1334.

MONGET, P., G. B. MARTIN (1997): Involvement of insulin-like growth factors in the interactions between nutrition and reproduction in female mammals. Human Reprod J. 12, 33-52.

NAOMAN, U. D. (2012): Effect of Ivermectin on semen characteristics of Iraqi Awassi ram. Al-Anbar J. Vet. Sci. 5 (2), 129-133.

ÕMURA, S. (2002): Macrolide Antibiotics - Chemistry, Biology and Practice. (Omura, S. Ed.), 2nd ed., Academic Press, pp. 571-576.

PARADIS, M. (1986): Ivermectin in small animal dermatology. In: Current Veterinary Therapy X: Small Animal Practice. (Kirk, R. W. ed.), Philadelphia, WB Saunders, pp. 560-562.

PAUL, A. J., W. J. TRANQUILLI (1986): Ivermectin. In: Current Veterinary Therapy X: Small Animal Practice. (Kirk, R. W., ed.), Philadelphia, WB Saunders, pp. 140-142.

PLUMB, D. C. (2002): Veterinary Drug Handbook, 4th ed., St. Paul, MN, Pharma Vet Publishing, pp. 454-458.

PURVIS, H. T., J. C. WHITTIER (1996): Effects of ionophore feeding and anthelmintic administration on age and weight at puberty in spring-born beef heifers. J. Anim. Sci. 74, 736-744.

SERI, H., I. HUSNA, M. ELBASHIR, O. P. IDRIS, T. HASSAN, O. Z. BARAKA (2000): Effect of Ivermectin on progesterone profile in the camel (*Camelus dromedarius*). The Sudan J. Vet. Res. 16, 17-22.

SUÁREZ, G., L. ALVAREZ, D. CASTELLS, O. CORREA, P. FAGIOLINO (2013): Relative bioavailability and comparative clinical efficacy of different ivermectin oral formulations in lambs. BMC Vet. Res. 9, 27-36.

TOUTAIN, P. L., D. W. UPSON, T. N. TERHUNE, M. E. McKENZIE (1997): Comparative pharmacokinetics of Doramectin and Ivermectin in cattle. Vet. Parasitol. 72, 3-8.

TURNER, M., J. M. SCHAEFFER (1989): Ivermectin and Abamectinn (Campbell, W. C., Ed.), Springer, New York, pp. 73-88.

WHITTIER, J. C., B. L. WEECH, M. C. LUCY, D. H. KEISLER, M. F. SMITH, R. M. CORWIN (1999): Effect of anthelminthic treatment on sexual maturation in prepubertal heifers. J. Anim. Sci. 77, 736-741.

WOODWARD, K. N. (2011): Toxicity in Animals: Target Species. Current Pharmacol. Biotechnol. 13, 952-968.

YELICH, J. V., R. P. WETTEMANN, T. T. MARSTON, L. J. SPICER (1996): Luteinizing hormone, growth hormone, insulin-like growth factor-I, insulin and metabolites before puberty in heifers fed to gain at two rates. Domestic Anim. Endocrinol. 13, 325-338.

SADEK, K. M., H. M. SHAHEEN: Biokemijski učinci ivermektina na spolne hormone i homeostazu minerala u krava baladi pasmine nakon teljenja. Vet. arhiv 85, 95-103, 2015.

SAŽETAK

Kod 20 zdravih i odraslih krava baladi pasmine, čija se tjelesna masa kretala od 250 do 350 kg, a dob od 4 do 7 godina, istražen je učinak antiparazitika ivermektina (IVM) na spolne hormone. Krave su bile podijeljene u dvije skupine (10 u svakoj skupini). Prvoj, kontrolnoj skupini bila je prvog dana nakon teljenja ubrizgana fiziološka otopina soli natrijeva klorida, a drugoj preporučena terapijska doza ivermektina (0,2 mg/kg, s/c). Uzorci krvi bili su prikupljeni 1., 15., 30. i 90. dana nakon primjene ivermektina. Rezultati su pokazali da je njegova primjena dan nakon teljenja dovela do odgode estrusa u trajanju do 3 mjeseca (izostali znakovi estrusa i strukture pri rektalnoj palpaciji jajnika). U razdoblju od 3 mjeseca došlo je do signifikantnog (P<0,05) sniženja razine folikulostimulirajućeg hormona (FSH), luteinizirajućeg hormona (LH) i estradiola u serumu. U istom razdoblju ivermektin je uzrokovao signifikantan (P<0,05) porast serumskog progesterona, prolaktina i kortizola, te slabo uočljive promjene razine serumskog testosterona i globulina koji veže spolne hormone. Razina kalcija u serumu značajno je porasla 1. dana, dok je razina fosfora u serumu signifikantno pala 15. i 30. dana od primjene ivermektina. Zaključeno je da ivermektin odgađa estrus kod krava u trajanju od 3 mjeseca putem narušavanja ravnoteže spolnih hormona odnosno poremećaja homeostaze kalcija i fosfora. Zbog toga se ne preporučuje njegovo davanje netom nakon teljenja. Nadalje, povišena razina kalcija nakon njegove prekomjerne primjene ne bi se smjela suzbijati davanjem kalcija, već upotrebom drugih antialergijskih pripravaka.

Ključne riječi: krave, ivermektin, homeostaza minerala, post partum, spolni hormoni