

# Juvenile hormone-like substances can induce vitellogenesis in the tick *Ornithodoros moubata* (Acarina : Argasidae)

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Topical application of different juvenile hormone analogs (JHA) or of a mixture of stereoisomers of insect juvenile hormone (JH) 1 and 3 to fed virgin female *Ornithodoros moubata* immediately after feeding induced vitellogenesis and egg-laying in up to 70% of treated females. In controls only 13.7% oviposited. The eggs were sterile, with abnormal shape, but their number versus the weight of engorged females was normal or sometimes greater than in mated females. However, preoviposition period was longer than in mated females.

It was more difficult to induce egg-laying by similar topical applications 100 days after feeding of virgin females. A maximum of 58% of ovipositing females was obtained with a very high dosage of JH mixture (500  $\mu$ g). Injection of this mixture into the females was more potent; 15 to 50  $\mu$ g induced oviposition in about 60% of the females. The preoviposition period was also longer than in control females.

Our results suggest the presence of a JH-like substance which is involved in the hormonal control of vitellogenesis. However, since natural isomers of JH were much less efficient than isomeric mixtures or JHA, we suppose that the natural tick hormone does not correspond to JH, but rather to a JH-like substance.

vitellogenesis; egg-laying; virgin females; tick; *Ornithodoros moubata*; juvenile hormone; juvenile hormone analogues

## Introduction

In many insects vitellogenesis is dependent on juvenile hormone (JH) which is secreted by the corpora allata (CA) [Reviews 1–3]. Mechanical (allatectomy or ligations) or chemical deprivation of CA (by means of precocenes which are anti-allatotropic [4]) prevents the secretion of JH and blocks oocyte maturation. Injection of JH, topical application of JH or Juvenile Hormone Analogues (JHA), or implantation of active CA is able to restore these processes in many species.

Vitellogenesis of the tick *Ornithodoros moubata* has been studied in this laboratory

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[5–8]. In virgin females the blood meal initiates vitellogenesis, but in the absence of copulation, digestion is arrested in most females and the developing eggs are resorbed. About 100 days after the blood meal, the ovaries look like those from unfed virgin females without any yolk proteins remaining in the haemolymph or in the oocytes. If these engorged virgin females are mated, digestion and vitellogenesis are resumed and egg laying occurs about 10 days after mating.

A few reports suggest a role for JH in the reproduction of argasid (soft) ticks. Diapause rupture and egg laying can be induced in fed mated female *Argas arboreus* by topical application of JHA [9]. However, oviposition was not induced in fed virgin females of this species. More recently, Pound and Oliver [10] were able to arrest ovarian development in *Ornithodoros parkeri* by treatment with the anti-alatotropic, precocene II, and restore it by topical application of JH3; however, reversal of the effects of precocene was not observed in *Argas persicus* [11].

Similar studies have been conducted on some species of the Ixodidae (hard ticks). In *Dermacentor variabilis*, Hayes and Oliver [12] supposed the presence of a JH during vitellogenesis. On the other hand, Mansingh and Rawlins [13] reported an inhibition of oviposition by different JHA in *Boophilus microplus*.

Thus, in view of these contradictory data, we investigated the possible effects of juvenile hormone-like substances in the control of vitellogenesis of the tick *Ornithodoros moubata*.

For this study, we used virgin females immediately after their blood meal, or more than 100 days after feeding, when the vitellus in oocytes is completely resorbed.

## Materials and methods

### *Animals*

Virgin *Ornithodoros moubata* (Murray, 1877; sensu Walton, 1962) females were obtained by isolation of fed fifth instar nymphs. These females were kept in the dark at 27°C and 30–40% relative humidity. Feeding was done on guinea pigs or with defibrinated pig blood through a 'Parafilm' membrane. These animals were used immediately after the blood meal or kept in individual glass vials for more than 100 days. In the last case, the few virgin females which had oviposited after the blood meal were discarded.

### *Chemicals*

Topical applications were performed with isomeric mixtures of JH1, JH2 and JH3 (Calbiochem), Farnesol (Fluka), Farnesyl methyl ether (FME) (gift of Dr. Beatrice Lanzrein, University of Bern) or SJ53C1 (3,7,11-trimethyl,11-chloro-2-dodecanoate ethyl ester) (synthesized by the Institute of Organic Chemistry and Biochemistry, University of Prague, and kindly provided by Dr. Sláma). JHs or JHAs were diluted in 1 µl acetone and applied dorsally. For injections, an isomeric mixture of JH1 and

JH3 'purum' (Fluka) or natural isomers of JHs (Eco-Chemical Intermediates) were used. In each case, 2.5  $\mu$ l of a solution of JH in olive oil were injected into the haemocoel through an articulation membrane of a leg with a microcapillary.

Controls with untreated fed mated females, untreated fed virgins, and treated fed virgins were run simultaneously for injection and for topical application experiments. No effects of the injections of oil or of the application of acetone was observed.

#### *Criteria for the activity of the tested compounds*

In the case of topical applications to newly fed females, only the females ovipositing within 30 days after the blood meal were considered as positive.

In the case of the injections into females 100 days after feeding, ovaries were dissected 25 days after the treatment and examined. Females considered as positive had ovaries containing oocytes larger than 200  $\mu$ m with a characteristic brown coloration due to the incorporation of the colored yolk proteins. This corresponds to the early stage III oocytes of Balashov [14], the beginning of the vitellogenesis. The genital ducts were also observed by means of the phase contrast microscope in order to attest the virginity of the females.

## **Results**

### *Oviposition in Ornithodoros moubata*

Vitellogenesis begins 4 days after feeding, and egg-laying starts after a preoviposition period of 10 to 13 days. During this 1st cycle of vitellogenesis, the number of oviposited eggs is directly proportional to the weight of engorged females 24 h after feeding (number of eggs =  $0.663 \times$  weight of engorged females - 21.422; correlation coefficient 0.94). This regression line was obtained with 82 fully engorged and 55 partially fed females. Sometimes a few engorged females (14.3%) lay non-viable eggs [7]. We have reinvestigated this percentage. Seven groups with a minimum of 15 females each, 187 individuals in all, were observed;  $13.7\% \pm 4$  oviposited a normal amount of eggs, proportionally to their engorged weight, after a preoviposition period of 10 to 30 days.

### *Effects of topical application of JHA and JH to fed virgin females*

As seen previously, fed virgin females generally fail to oviposit. We have therefore investigated the effects of topical application of JHA and JH on these females immediately after the blood meal or 100 days after feeding.

#### *(a) Application immediately after the blood meal*

Virgin females were fed and afterwards received different doses of JHA or JH. In each case, this treatment induced egg laying in the females (Fig. 1). However, never

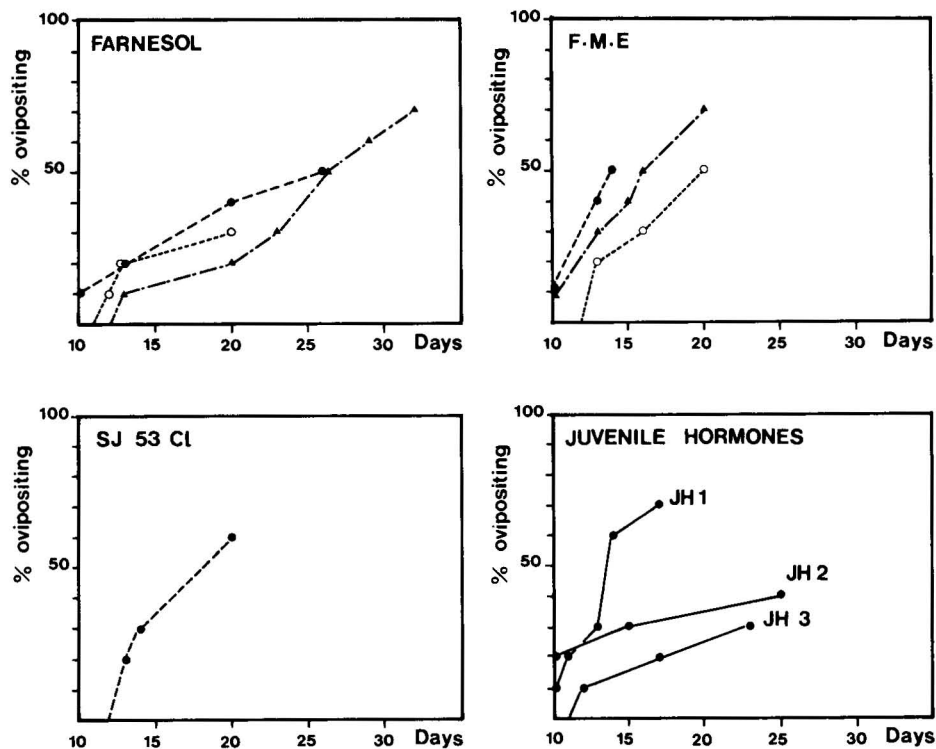


Fig. 1. Cumulative percentages of ovipositing virgin females following topical application of JHAs and JHs stereoisomers immediately after the blood meal, in relation to their preoviposition time in days. ○·····○, 1 µg per female; ●-----●, 10 µg per female; ▲- - - -▲, 100 µg per female. 10 µg of stereoisomeric mixtures of JH1, JH2 and JH3 were applied per female.

more than 70% of the females oviposited (no correlation existed between individual tick weight and oviposition). The preoviposition period was often longer than normal (10 to 13 days), but this delay of oviposition differed among the substances. In the case of farnesol, the higher the dose tested, the greater was the percentage of egg-laying females.

Farnesyl methyl ether (FME) induced the same effects, but the influence of dosage appeared less evident. However compared with farnesol, FME was able to provoke egg laying more rapidly. At the highest dosage, 70% of the females began to oviposit within 10 to 20 days after the blood meal. Approximately the same percentage (60%) and the same preoviposition period was observed with the JH analogue SJ53C1.

Topical applications of 10 µg of synthetic isomeric mixtures of JH were also tested. JH1 appeared to be the most efficient, viz., 70% of these females oviposited within 17 days. Isomeric mixtures of JH2 and of JH3 were less active than JH1 or the JHA tested previously; comparatively low percentages (respectively 40 and 30% of the females oviposited) were obtained at the end of the experiment with a greater

preoviposition period than with JH1. Eggs obtained during these experiments were sterile and became desiccated a few weeks after oviposition. In addition, their shape was often oblong instead of spherical.

In order to compare the efficiency of egg production in treated females with that of control mated females, the number of eggs laid was counted and correlated with the weight of the engorged females after 24 h (Fig. 2). In the case of farnesol and of SJ53C1 the number laid was not significantly different from control mated females. On the other hand, FME increased the number of eggs laid per mg of engorged females. The maximum increase was obtained with 10  $\mu\text{g}$  per animal. JHs, especially JH1 also seemed to increase the efficiency of egg laying.

(b) Application 100 days after feeding

Similar experiments to those described above were performed on virgin females

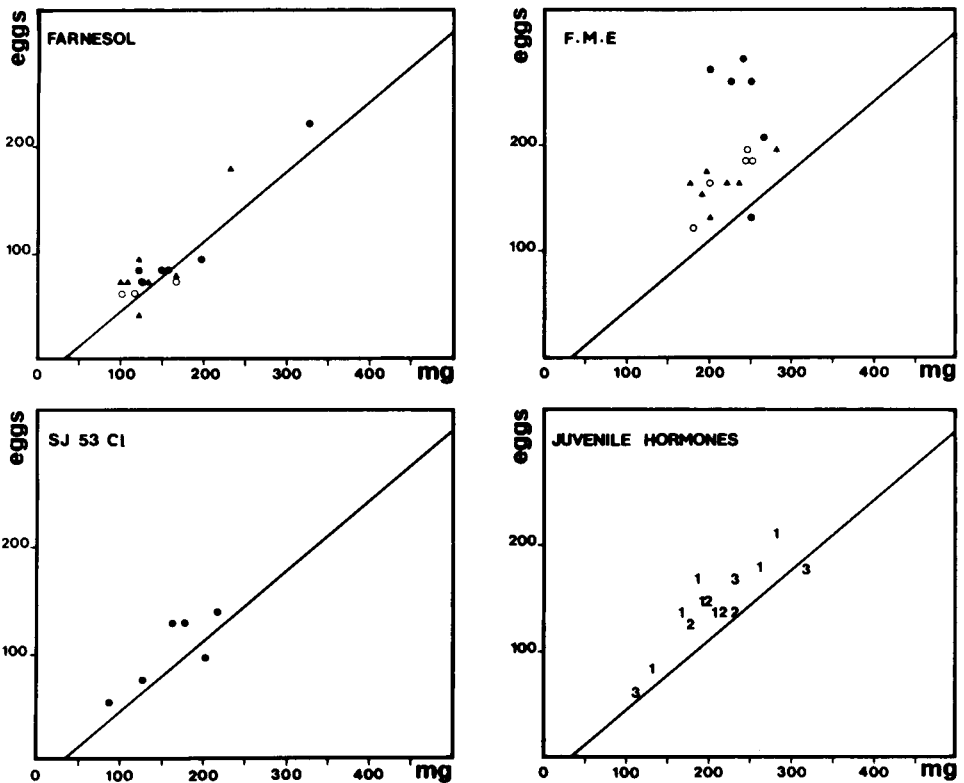


Fig. 2. Egg yield in virgin females following topical application of JHAs or JH stereoisomeric mixture immediately after the blood meal. Normally, mated females *O. moubata* oviposit a number of eggs proportional to their engorged weight 24 h after the blood meal and the corresponding regression line is included in each of the 4 diagrams. Experimental values for the different doses of substances tested: juvenile hormone analogues:  $\circ$ , 1  $\mu\text{g}$ ;  $\bullet$ , 10  $\mu\text{g}$ ;  $\blacktriangle$ , 100  $\mu\text{g}$ ; juvenile hormones 1, 2 and 3: 10  $\mu\text{g}$  in each case.

TABLE I

Effects of topical application of Juvenoids to virgin females *O. moubata* fed 100 days previously

Substance	No. of treated females	Percentage of egg-laying females	Time of preoviposition
Farnesol			
10 $\mu\text{g}/\text{female}$	15	20	12–34 days
FME			
10 $\mu\text{g}/\text{female}$	15	20	10–16 days
SJ 53 C1			
10 $\mu\text{g}/\text{female}$	10	40	10–28 days
Mixture JH1–JH3			
1 $\mu\text{g}$	15	33	
50 $\mu\text{g}$	15	27	17–20 days
500 $\mu\text{g}$	12	58	

100 days after the blood meal. In such females, the vitellus is completely resorbed, and copulation is usually necessary to initiate a complete gonotrophic cycle.

Table 1 summarizes the results obtained with the different substances. With farnesol and FME (10  $\mu\text{g}/\text{animal}$ ) only a small proportion of ovipositing females was obtained (20%). This proportion was very close to the normal proportion obtained in control females treated with acetone. SJ53C1 provoked a slightly higher percentage of egg laying (40% with a preoviposition period of 10 to 28 days).

A stereoisomeric mixture of JH1 and JH3 was also tested at 3 different concentrations (1, 50, and 500  $\mu\text{g}/\text{animal}$ ). The first 2 dosages induced egg laying in approximately 30% of the females, the highest dosage (500  $\mu\text{g}$ ) was more efficient and provoked oviposition in 58% of the females.

#### *Effects of injection of insect juvenile hormone into virgin females 100 days after feeding*

Since the highest dosages of topically applied isomeric mixtures JH1–JH3 were most potent upon egg laying, we investigated the effects of injected (natural and

TABLE II

Injection of a mixture of stereoisomers of JH1–JH3 (1:1) into virgin female *O. moubata* fed 100 days previously

Dose ( $\mu\text{g}$ )	No. of injected females	Percentage of positive females
1	11	27
15	10	60
50	28	57
150	10	40
500	14	Death

TABLE III

Injection of stereoisomers of JH1 and JH3 into virgin female *O. moubata* fed 100 days previously

	No. of females	Vitellogenesis after 10 days (%)	Vitellogenesis after 25 days (%)
JH 1 25 $\mu$ g	27	10	18
JH 1 75 $\mu$ g	26	30	50
JH 3 25 $\mu$ g	25	30	20
JH 3 75 $\mu$ g	29	40	37

Some of the females were dissected after 10 days and the remainder after 25 days. The ovary was observed for the presence of vitellogenesis and of ovulated eggs.

isomeric synthetic mixtures) insect JH. With this technique, the cuticular barrier was avoided and the substance could presumably reach the target tissue more easily.

Table 2 gives the results of increasing dosage of injected isomeric mixture of JHs. At the dose of 15  $\mu$ g or 50  $\mu$ g, approximately 60% of the females laid eggs; egg laying began within 17 to 23 days. Higher dosages seemed to decrease the percentage of ovipositing females. 500  $\mu$ g was a lethal dose and females died within 3 to 5 days of injection.

The delay of oviposition in the treated females could be explained by a delay in the onset of vitellogenesis. Table 3 shows that 10 days after injection only a few females showed signs of vitellogenesis upon dissection, whereas after 25 days the

TABLE IV

Effects of injection of natural insect juvenile hormone 1, 2 and 3 into virgin female *O. moubata* fed 100 days previously

Dose ( $\mu$ g)	No. of females	Percentage of vitellogenic females	No. ovipositing within 25 days
JH1			
0.1	15	14	1
1	14	7	1
10	14	7	0
100	14	7	0
JH2			
0.1	14	7	0
1	14	7	0
10	13	8	1
100	14	36	4
JH3			
0.1	12	0	0
1	13	0	0
10	14	7	0
100	14	21	2

The ovary was observed after 25 days for presence of vitellogenesis.

proportion had greatly increased. 50 and 37% of the females injected with 75  $\mu\text{g}$  JH1 and JH3, respectively, were vitellogenic. This experiment also seems to indicate that JH1 was more effective than JH3.

Thereafter, other experiments were performed with natural isomers JH1, 2 and 3 (Table 4). Only the 100  $\mu\text{g}$  dosages of JH2 and of JH3 may be considered effective since 36 and 21%, respectively, of females oviposited. However, natural isomers of JHs were less potent than mixtures of isomers of JHA.

## Discussion

The control of vitellogenesis in insects has been studied extensively during the last decade [1–3,15]. In many species, the role of juvenile hormone is well established; e.g. in *Rhodnius prolixus* and in *Locusta migratoria* JH acts on two levels: (1) to induce synthesis of vitellogenin by the fat body and (2) directly on the ovary to permit the uptake of the proteins [16,17]. However, in the Diptera, ecdysteroids alone [18,19] or together with JH [20] seem to play a role in the control of vitellogenesis, although these interpretations are still a matter of debate [21].

Two reviews [8,22] report the work which has been done on vitellogenesis of ticks. As in numerous haematophagous species, the blood meal is necessary for the induction of vitellogenesis. In addition, in most ticks, copulation is also important for successful egg maturation. As in other animal species, oogenesis probably requires more than one hormone. However, these hormones have yet to be identified.

Exogenous applications of JHA and JH were performed on fed virgin female *O. moubata* in order to explore a possible function of these hormones in ticks. Our experiments clearly demonstrate an effect of these substances on vitellogenesis since some of the substances induced egg laying in a high percentage of females, while in untreated fed virgins, vitellogenesis was abortive. The number of eggs produced by treated females was as high as the number expected for mated females of the same weight. In addition, in the case of the highly active FME, with a dose of 10  $\mu\text{g}$  per female, the egg-yield was clearly higher than in control mated females. Our results with freshly fed virgins demonstrate that the action of JH or JHA is to maintain or to hyperstimulate (FME) vitellogenesis which is initiated by the blood meal. In addition, we could show that fed virgins which have completely resorbed their eggs of the first abortive gonotrophic cycle, can resume complete oogenesis. This indicates that these compounds can also initiate the vitellogenic process. Consequently, we suggest that a hormone comparable to JH of insects plays a role in the control of vitellogenesis of *O. moubata*.

It also appears that vitellogenesis and blood meal digestion are closely linked and that the hypothetical hormone may also play a role in the control of digestion, since in virgins fed 100 days previously, resumption of vitellogenesis is not possible until digestion is resumed. Thus, we can postulate that the blood meal may trigger a preliminary burst of 'JH' secretion which could be responsible for the initiation of digestion and subsequently vitellogenesis, while stimulation by copulation is required to maintain the hormone production necessary for completion of digestion and

oogenesis. If the fed females are not mated, vitellogenesis is abortive. In such females, 'JH' which presumably is produced after stimulation of a delayed copulation serves both to initiate and to maintain a complete cycle of digestion and vitellogenesis.

Other related works on argasid ticks seem to indicate also a role for JH during vitellogenesis. JHA topical application to mated fed female *Argas arboreus* induced diapause rupture and subsequently resumption of the gonadotrophic cycle [9]. However, in this case, it is not clear if oviposition is a consequence of JH treatment or of other gonadotrophic hormones released after diapause rupture. In *O. porcinus porcinus*, topical application of 10  $\mu\text{g}$  of the natural *cis*, *trans*, *trans* isomers (stereochemical purity greater than 85%) of JH1 and JH3 to virgins 2 days after feeding induced oviposition in a few females (28%) while there was no oviposition among JH2 treated females [22]. This contrasts with our findings in *O. moubata* where JH2 alone was slightly effective.

In *O. parkeri*, vitellogenesis was inhibited by precocene II which prevented JH synthesis and thus vitellogenesis as in many insects. Furthermore, reversal of this effect was obtained by topical application of JH3 [10]. However, reversal of the effects of precocene was not possible in *Argas persicus* [11].

In conclusion, we recognize that the precise character of the hormone involved in the control of vitellogenesis in argasid ticks has yet to be determined. However, our results demonstrate, for the first time, that JHs or JHA can elicit vitellogenesis in fed virgin females. Not all the females oviposited, and the preoviposition period was prolonged, but similar observations have also been reported with respect to JH-treated, allatectomised insects where JH is known to be the natural hormone [23,24].

Our results, obtained with large doses of JHs or JHAs do not show that we are dealing with a direct effect, but suggest, along with the results obtained with *O. parkeri* [10], that a hormone structurally related to JH may exist in these animals. Future studies should be aimed at the isolation and chemical identification of the natural hormones playing a role in the control of vitellogenesis of argasid ticks.

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