

# Reproductive disorders in a laboratory colony of *Glossina palpalis palpalis* Robineau-Desvoidy (Diptera : Glossinidae)

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AHMED (A.B.), ONYIAH (J.A.), SULEIMAN (S.N.). Reproductive disorders in a laboratory colony of *Glossina palpalis palpalis* Robineau-Desvoidy (Diptera : Glossinidae). *Revue Élev. Méd. vét. Pays trop.*, 1995, 48 (3) : 259-263.

Reproductive disorders were used as a framework in assessing the loss in fecundity of a colony of *G. p. palpalis*. One thousand five hundred females at various stages of the pregnancy cycle were selected at random from the main colony and divided into 10 groups of 150 individuals each according to age, which ranged between 5-90 days. All the groups were observed for the next 30 days and then dissected. The result showed that abortions were the commonest form of disorder and the major source of loss of fecundity, closely followed by the pupariation of third instar larvae *in utero*. The incidence of ovular blockage, insemination failure and degeneration of embryo/egg follicles was low. No instance of atrophy of the ovary was seen. Aborted eggs and larvae were recorded from all the age groups studied, indicating that abortions occur at any stage of the female pregnancy cycle. With a total reproductive disorder of 3.0 %, losses in fecundity through reproductive abnormalities in the tsetse colony studied are minimal.

*Key words* : *Glossina* - Reproductive disorder - Abortion - Nigeria.

## INTRODUCTION

Unlike most insects of major veterinary and medical importance, tsetse flies (*Glossina* spp.) have a unique mode of reproduction characterised by adenotrophic viviparity. Fertilised eggs and the resulting larvae are retained within the uterus, fed by the nourishment from the milk gland (6, 28) and deposited one at a time at regular intervals. Under our maintenance conditions, the first larva is produced between day 18 and 20 post emergence and subsequent births are at regular intervals of between 9-11 days (1).

Despite this low reproductive potential, offspring numbering as many as 20 have been recorded from a single female tsetse that lived for almost 200 days in an optimum laboratory environment (3). Under natural condi-

tions, however, a lower number of offspring are produced owing to several constraints associated with independent existence.

Information on the reproductive status of female tsetse was used to measure the stability of the tsetse population under natural conditions (31). Such information can also be used in assessing the reproductive performance of laboratory colonies. Saunders and Phelps (27) reported that abortion, egg retention and follicular degeneration are the commonest disorders and main sources of reproductive losses in tsetse laboratory colonies and the result of later studies on *G. p. gambiensis* (29) is not very different. In this paper, the reproductive disorders of female tsetse flies from a self-producing colony of *Glossina p. palpalis* was evaluated to identify the main reproductive disorders and the sources of reduced fecundity amongst females in the colony.

## MATERIAL and METHODS

One thousand five hundred producing female *G. p. palpalis* Robineau-Desvoidy at various stages of the pregnancy cycle were selected at random from the colony kept at the main tsetse vector laboratory of the Nigerian Institute for Trypanosomiasis Research (NITR) previously described by Onyiah *et al.* (21) and divided into 10 groups according to age. Male flies were used more than once for routing mating. Each group consisted of 150 individuals. Flies were placed 10-20 per plastic cage (18 × 8 × 4 cm), maintained in the same room as the main colony and observed for 30 days. A second group of 91 flies consisting of only gravid females was chosen from the main colony and used specifically to study the relationship between *in utero* pupariated mortality and mortality by other causes.

Larviposition trays were checked daily during routine collection of puparia and any abortion seen was recovered, classified and recorded. Flies dying before the end of the observation period were dissected and the condition of the spermathecae, ovary and uterus were examined using the method of Saunders (25). Surviving flies were dissected on day 30 post grouping and their reproductive status was determined. For all flies, the occurrence of the following disorders was investigated : ovular blockage (egg retention), abortion, follicular/embryonic degeneration, pupariation of third-instar larvae *in utero* and atrophy of the ovaries.

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## RESULTS

Table I sums up the reproductive status of the representative female population in the tsetse colony studied. Only six females in group 1 examined were not inseminated, giving an overall insemination rate of 99.6 % ; four had mating scars, indicating that they had copulated, while the remaining two were complete virgins.

It is common for flies to abort a pregnancy after which they successfully carry subsequent ones to full time. Since abortions do not leave any traces, later dissection of such females after passing through successful cycles would not reveal evidence of the previous abortions. For this reason, only the abortions physically recovered were used in estimating the loss of fecundity in this study.

A total of 28 abortions were recovered and classified (table II). From the total number of flies dissected at the end of the observation period, eight females showed an empty uterus and immature eggs next in the sequence of development, which is an indication that they had aborted their pregnancies. These were however not included in the total estimate on the assumption that the aborted pregnancies had been recovered previously as shown in table II. 95.9 % of the 73 (4.7 %) females with an empty uterus had fully developed eggs next in the ovulation sequence, indicating recent parturitions.

Ovular blockage (egg retention) showing developed eggs in most of the 4 ovarioles was observed in 2 of the 6 non-inseminated females ; the two females were virgins. One female with a degenerating egg follicle in one of the ovarioles was observed in group IV ; a further three from groups III, V and VIII were observed with empty egg shells in the uterus, indicating embryo degeneration.

TABLE II  
Abortions recovered in a representative female colony of *G. p. palpalis*

Sample size	Egg	Larval instars			Total
		I	II	III	
1,500	12	4	7	5	28

Seven flies died with pupariated third instar larvae within the uterus (with 1/3 of these number half way through birth). Considerably higher mortality was recorded with increasing age among the flies ; more than 75 % of the 596 flies that died before the end of the observation period were older than 80 days.

## DISCUSSION

Despite the fact that the sample population studied showed evidence of copulation and spermathecal filling, the results revealed an overall reproductive disorder of 3.0 % comprised of 1.9 % abortions, 0.5 % uterine pupariation, 0.4 % non-insemination, 0.2 % embryo degeneration and 0.1 % each of ovular blockage (egg retention) and follicular degeneration respectively.

Since virgin females with evidence of copulation (mating scars) were found only in group I, it is most probable that some of the males used for the routine mating were mixed up and those used for that group were either too young or had not fully recovered from the effects of previous matings. Foster (12) reported that male tsetse are not fully potent before day 5 post emergence when their

TABLE I  
Reproductive status in a representative female *Glossina p. palpalis* laboratory colony

Fly group	Age post emergence DAY + 30	Mating scars (%)		Spermathecae (%)		Uterine disorder (%)			Ovarian disorder (%)	
		+ ve	- ve	+ ve	- ve	Empty	EMD	UPP	OBL	FD
I	35	98.7	1.3	96.0	4.0	0.7 (1.3)	—	0.7	1.3	—
II	48	100	—	100	—	14.0 (0)	—	1.3	—	—
III	57	100	—	100	—	6 (0.7)	0.7	—	—	—
IV	66	100	—	100	—	0.7 (1.3)	—	—	—	1.3
V	75	100	—	100	—	2 (0)	0.7	0.7	—	—
VI	84	100	—	100	—	1.3 (0.7)	—	—	—	—
VII	93	100	—	100	—	0 (0)	0.7	—	—	—
VIII	102	100	—	100	—	4 (0.7)	—	0.7	—	—
IX	111	100	—	100	—	12.7 (0.7)	—	0.7	—	—
X	120	100	—	100	—	7.3 (0)	—	0.7	—	—
<b>Total</b>		<b>99.9</b>	<b>0.1</b>	<b>99.6</b>	<b>0.4</b>	<b>4.9 (0.5)</b>	<b>0.2</b>	<b>0.5</b>	<b>0.1</b>	<b>0.1</b>

+ ve = positive ; - ve = négative ; EMD = embryo degeneration ; UPP = uterine pupation ; OBL = ovular blockage ; FD = follicular degeneration. Figures in brackets indicate empty uterus as a result of abortions observed through dissection.

accessory glands have fully developed following consecutive blood uptake and Saunders and Dodd (28) demonstrated that repeated use of males for mating without an adequate rest period renders them aspermic. From these results, it is apparent that in practical terms, the appearance of mating scars alone should not be used as an index of insemination.

Abortion is the commonest form of reproductive disorder in the present study. Similar results have been recorded by other workers both in the laboratory (18, 27, 29) and in natural populations (17, 32). Besides implicating inadequacy of food and interruption in feeding as the major causes (18), climatic stress (20, 35) and physical handling of pregnant females have also been suggested as causative factors. The presence of antibiotics and other additives in the diet of the animal hosts used in fly feeding is also known to cause abortion in tsetse flies (14, 26, 33). In this laboratory, handling is done manually and is therefore likely to impose greater stress on the flies than the influence of climatic factors which remained stable through the use of effective control equipment; the possibility of additives in the host diet acting as toxicants to the flies (14, 26, 33) has also been eliminated following the adoption of a locally-formulated-additive free diet for the animals (2).

The result further suggests that abortion amongst females occurred in all the stages of the gestation period, which is in agreement with the suggestion of Saunders and Phelps (27). The occurrence of these abnormalities in all the age groupings however failed to lend support to the suggestion of Glasgow (13) of an age-related reduction in fecundity of females from increased abortion among older flies. Similar results to those observed in the present study were observed in natural tsetse populations (17, 20).

While the possibility of seeing flies in conditions between parturition and ovulation in natural tsetse populations is considered remote in view of the short time interval between the two processes (20), the majority of the flies encountered with empty uteri in the present study had evidently larviposited shortly before they were killed for dissection. This is not surprising because the flies were killed between 0800-1000 hours on the day when they were dissected and more than 30 % of the parturitions in this laboratory occur between 0600 and 1200 hours (A.B. Ahmed, manuscript in preparation).

In this study, *in utero* pupariation of third instar larvae evidently occurred in most of the fly groups irrespective of age. Generally, the literature on the occurrence of this disorder in *Glossina* species is scanty (19, 23, 29, 30). The only known attempt to explain the likely cause of this disorder is that of Nash *et al.* (19), who observed a strong relationship between engorgement shortly before parturition by pregnant females and the frequency of occurrence of the anomaly, and suggested that the complex of pressures which develops following the blood intake forces

the polypneustic lobes out of the vulva, changing the normal larval lying position and making extrusion difficult. Later observation (4) in a similar experimental set-up did not confirm the above. Tobe and Davey (32) noted that the size of the blood meal ingested by pregnant females is regulated by the available space within the abdomen and it has been shown (6,15) that the last meal is mostly taken about 72 hours prior to parturition. During the present investigation, observation of 91 gravid females fed daily and simultaneously on the same hosts through three cycles also failed to reveal any difference between the proportion of females dying with pupariated larvae within their uterus and those dying of other causes.

In attempts to understand the control of parturition, several experiments conducted (5, 7, 11, 22) have demonstrated the involvement of the nervous and endocrine systems of the female. Studies by Denlinger (5) showed that parturition rhythm can be triggered by either mother or larva and presumed that the two contribute in determining the timing.

The importance of timing of parturition and integration of other reproductive events in *Glossina* species has already been emphasized (6). The factors responsible for pupariation within the maternal uterus cannot be readily explained. On the basis of our present knowledge of the mother-larva relationship, it seems that the anomaly is associated with a breakdown in coordination between mother and offspring on the timing of the commencement of parturition. This suggestion is strengthened by the observation that whether parturition is triggered or not, further pupariation events proceed unhindered since the various tissues of the third instar larva become committed to metamorphosis while still in the uterus following ecdysteroid induction (8). Dzadrek and Denlinger (10) showed that manipulation of the larva including wrapping in paraffin and wedging into narrow plastic cones (which corresponds to the confines of the maternal uterus) causes acceleration of pupariation and that the timing of the different events remained fixed. Furthermore, since pupariation *in utero* occurred either wholly within the uterus or half way through birth, could it be that the former results from total failure at initiating the parturition and the latter from a delay in completing the already triggered process? Yet again, Denlinger and Dzadrek (9) reported that the actual expulsion of the larva is achieved within a short period of 5-12 seconds. Further study is needed to understand this complicated but vital aspect of tsetse reproduction.

Instances of ovular blockage (egg retention) were recorded only among the non-inseminated females who apparently had not copulated, in view of the absence of mating scars on their bodies. This lends support to the observation of Saunders and Dodd (28) on *G. morsitans* that the act of mating is an essential prerequisite for normal ovulation of the first egg. Mellanby (18) similarly observed several eggs within the ovaries of unmated virgin female flies.



Results from the present study also revealed a very low incidence of both *in utero* degenerating embryos and degenerating follicles within the ovaries. Prolonged high environmental temperature may not be the sole causative factor as earlier suggested (18, 24). The role of other unknown factors cannot be ruled out since the conditions under which flies are maintained in this laboratory are optimal. The occurrence of almost all types of reproductive anomalies associated with colony flies and the absence of positive correlation between some of the disorders observed in this study and their documented causes described in the literature may have been due to the effects of the endogenous, physiological factors such as developmental aberration and hormonal imbalance proposed by Turner and Snow (34). The overall low level of reproductive disorders indicates a healthy reproductive system. In the light of the above results, it is concluded that the reduction in productivity through reproductive disorders of female tsetse flies under the prevailing laboratory conditions is minimal.

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AHMED (A.B.), ONYIAH (J.A.), SULEIMAN (S.N.). Troubles de la reproduction dans un élevage en laboratoire de *Glossina palpalis palpalis* Robineau-Desvoidy (*Diptera* : *Glossinidae*). *Revue Elev. Méd. vét. Pays trop.*, 1995, **48** (3) : 259-263.

Les troubles de la reproduction ont été utilisés comme paramètre pour l'évaluation de la baisse de fécondité d'une colonie de *G. p. palpalis*. Mille cinq cents femelles, parvenues à différents stades du cycle de la gestation, ont été choisies au hasard au sein de la colonie principale et réparties en dix groupes de 150 individus chacun en fonction de leur âge compris entre 5 et 90 jours. Tous les groupes ont été soumis à une observation au cours des 30 jours suivants et, au terme de cette période, les glossines ont été disséquées. Les résultats ont montré que l'avortement représentait la forme la plus courante des troubles de la fonction de reproduction et la cause principale de la baisse de la fécondité, suivi immédiatement par la pupaison *in utero* des larves parvenues au troisième stade larvaire. L'incidence du blocage ovulaire, de l'échec de l'insémination et de la dégénérescence des follicules de l'embryon ou de l'œuf était faible. On n'a observé aucun cas d'atrophie de l'ovaire. Les avortements d'œufs et de larves ont été enregistrés dans toutes les classes d'âge étudiées, ce qui indique que l'avortement survient à n'importe quel stade du cycle de gestation des femelles. Les troubles de la reproduction ne dépassant pas 3 p. 100 au total, la baisse de la fécondité due à des anomalies de la reproduction dans la colonie de tsé-tsé étudiée est minime.

Mots-clés : *Glossina* - Trouble de la reproduction - Avortement - Nigeria.

AHMED (A.B.), ONYIAH (J.A.), SULEIMAN (S.N.). Desórdenes reproductivos en una colonia de *Glossina palpalis palpalis* Robineau-Desvoidy (*Diptera* : *Glossinidae*) en laboratorio. *Rev. Elev. Méd. vét. Pays trop.*, 1995, **48** (3) : 259-263.

Los desórdenes reproductivos se utilizaron como marco para confirmar la pérdida de fecundidad de una colonia de *G. p. palpalis*. A partir de una colonia principal, se seleccionaron al azar 1 500 hembras en diversos estadios del ciclo de preñez y se dividieron en 10 grupos de 150 individuos, de acuerdo a la edad, variando entre 5 y 90 días. Todos los grupos se observaron durante los siguientes 30 días y luego fueron disecados. El resultado demostró que los abortos constituyen el desorden más frecuente y la principal fuente de pérdida de fecundidad, seguidos de cerca por la puparización *in utero* de larvas de tercer estadio. La incidencia de bloqueo ovular, el fracaso de la inseminación y la degeneración de folículos embrión/huevo fue baja. No se observó atrofia de ovario. Se observaron los huevos abortados y las larvas de todos los grupos estudiados, indicando que el aborto se presenta a cualquier edad del ciclo de preñez. Con un total de desórdenes reproductivos de 3 p. 100, las pérdidas de fecundidad provocadas por anomalías del ciclo reproductivo de las colonias tsetse estudiadas fueron mínimas.

Palabras clave : *Glossina* - Trastorno de la reproducción - Aborto - Nigeria.