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BARD

FINAL REPORT

PROJECT No. I-115-79

Endocrine Control of Prolapsed Oviduct in Hens

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1983

BARD
P. O. Box 6
Bet Dagan, Israel

March 30, 1983

BARD Project No. I-115-79

Title of Research Project:

Endocrine control of prolapsed oviduct in hens

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Project's starting date: April 1, 1980

Type of Report: Initial _____ Comprehensive _____ Final X

Signatures:

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* Final report submitted by Laurence Shore, Ph. D. who was responsible for the last year of the grant.

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Acknowledgements

We would like to thank Drs. Bendheim and Totach of the Poultry Disease Laboratory in Jerusalem who performed the field experiments. We would also like to thank our technical staff at Beit Dagan, A. Ailenberg, Sarah Lavi and F. Mileguir, for their co-operation and Dr. Y. Weisman for his assistance and advice. Finally, we would like to thank the technical staff at BARD for their flexibility and co-operation in managing this grant.

Note: Although this is the Final Report, BARD has allowed an extension of the grant till Sept. 1983 so that more field experiments can be performed.

Abstract

Prolapsed oviduct in the hen causes a substantial number of deaths in flocks in Israel. In the course of this investigation, we attempted to find a treatment for affected coops and to determine the underlying biochemical deficiencies of this syndrome. It was found that a program of aggressive management of early identification and isolation of affected birds and the treatment of more serious cases with physiological amounts of exogenous estrogen could reduce the mortality in affected coops from 2% to less than 0.1% within three weeks of treatment. Reducing the light intensity or debeaking was marginally effective. As to the biochemistry of the syndrome, it was found that prolapsed hens had considerably lower plasma estrogen concentrations as the result of reduced estrogen synthesis by the ovary. This reduced estrogen synthesis was related to lower activity of prostaglandin synthetase in the oviduct and the post-ovulatory follicle. The deficiency in prostaglandin resulted in poorer contractility with increased intra-abdominal pressure which causes the prolapse. As to the etiology of the syndrome, it was found that anti-estrogens or pharmacological doses of estrogen exacerbate the syndrome. This indicated that exogenous estrogenic substances in the environment are a possible factor in this syndrome.

Introduction

Prolapsed oviduct is a common problem familiar to all poultry farms but has received scant attention in the scientific literature. This may be because that until recently it has not been a problem of great economic importance or that it was thought to be untreatable. Although some textbooks have made passing reference to the syndrome with some speculation as to the possible cause (infection, excessive fat), the only attempts at scientific investigation seem to be that of Burmester (1948) who claimed to cause prolapse with testosterone injections and Meronuck et al (1970) who demonstrated that prolapse can be caused by some substance found in *Fusarium roseum* infected corn. The problem in Israel in the past few years has however been quite severe with many coops having mortality rates of 2% or more a month and several coops have become uneconomical and eliminated solely due to prolapse. We therefore in this project attempted to gain an understanding of the basic biochemical and physiological characteristics of this syndrome as well as find an effective method of reducing mortality and morbidity rates to acceptable levels.

A. Description of the syndrome

1. Pathology

Prolapsed oviduct in the chicken is usually defined as the eversion of the oviduct with the cloaca through the vent. More strictly it is the failure of the uterus to be drawn back normally after oviposition. In its most mild form, there are blood stained eggs and examination of the cloaca by manually increasing the intra-abdominal pressure shows an inflamed or eroded vagina (termed suspect). In more severe cases, several centimeters of oviduct are seen externally and there is a copious white (presumably due to uric acid) excretion from the cloaca. In the most acute form "blow-out", there is a sudden expulsion of the oviduct, cloaca and intestines. This form is invariably fatal within a few hours but is not very common as it was seen in less than 2% of birds displaying a prolapsed oviduct. Death occurs due to cannibalism, i.e., other birds peck it to death; uremia, probably due to mechanical blockage of the ureter; internal ovulation into the abdominal cavity with resultant serositis; or intestinal strangulation. As near as can be determined, mortality is 100% if birds are left untreated. Prolapsed oviduct per se however is not necessarily fatal as evidenced by one exceptional layer which was observed in prolapse for over a year and a half.

Prolapsed oviduct has been reported in all areas of Israel*. Affected coops were both trap nests and batteries. It was found in all of the commercial strains in use in the areas we observed. (These strains were various crosses between white Leghorns and Rhode Island reds.) Peak mortality was observed in birds 5 to 8 months old regardless of the time of year. The experiments reported here took place on Beit Meir and Machsiya in the Jerusalem hills, Hafatz Haim in the central plains, and Maagan in the lower Galilee.

B. Environmental factors and treatment protocols

1. Effect of light.

The effect of light intensity was studied in three farms (Tables 1,2,3). In each case, there was reduced incidence and mortality of prolapsed hens on the side receiving less light (less than 100 lux) than on the more brightly lit side (greater than 500 lux). However, field experiments with reducing the light were inconclusive and benefit if any was marginal. The effect of shortened photoperiod was studied in two coops with controlled lighting in Bet Dagan. Birds were kept in individual cages and nearly all birds (90%) recovered regardless of photoperiod. Reducing the photoperiod did however reduce the time for recovery from about 30 days to about 20 days (Table 4). Essentially none of the birds reprolapsed (2%) during the six months following recovery.

* We have had informal reports of the syndrome being of concern in Arizona and Cyprus and it was apparently common in Eastern Europe in the last century.

B-2. Effect of pharmacological doses of estrogen

Groups of birds, 8-10 in a group, received 450 μ g of estradiol (50 μ g three times a week for three weeks). One group of 6 received 900 μ g and one group 90 μ g on the same schedule. The criteria for recovery was that the birds recovered from prolapse within three weeks. It was found that with (6/16) or without injections (8/20) of estradiol, 40% of the birds did not recover when exposed to short photoperiods (6 L:18D, light:dark). However, while untreated birds exposed to long photoperiods (20:4) also had a 40% rate of non-recovery, 85% (17/20) birds receiving estrogen injections were still in prolapse at the end of the observation period. In the group of 6 receiving 900 μ g of estradiol, 3 of the birds had blowouts after they had partially recovered. 90 μ g of estradiol was found to be as deleterious as the 450 μ g dose. In a different experiment, under field conditions, it was observed that four birds that received tamoxifen, a potent anti-estrogen, did not recover during the one month they were isolated.

Injections of pharmacological doses of estrogen into healthy hens had no observable clinical effect other than to perhaps raise the rate of lay by some 5%. However there were biochemical abnormalities similar to that seen in the prolapsed hen and these will be discussed in section C. Injections of tamoxifen or clomiphene citrate (anti-estrogens) also did not provoke prolapse but did appear to delay the time of ovulation and thereby reduce the rate of lay slightly.

B-3. Effect of exogenous testosterone

Burmester in his 1948 paper claimed that injections of testosterone and possibly estrogen could cause prolapse in healthy chickens. We attempted a number of times to repeat this experiment but doses of testosterone (1 mg/3x/3wk) sufficient to cause hens to cease laying and evoke signs of masculinization were ineffective in causing prolapse even in hens with a previous history of prolapse.

B-4 Effect of physiological doses of estrogen

In a series of four field experiments, 17 hens received 100 ng of estrogen three times a week for three weeks or shorter if they recovered before then. 16/17 hens recovered fully and returned to normal lay within 11.5 ± 1.5 days. During the same three weeks in field experiments, 3 of the 11 controls which received corn oil alone died and 1 recovered. In one experiment, 4 birds which received tamoxifen (1mg/ml) did not improve during the three weeks of injection but did recover when subsequently treated with estrogen. In most of the experiments the investigator was unaware whether he was giving estrogen or corn oil alone.

B-5 Effect of isolation and aggressive management

In three coops of about 6000 hens with mortality rates of more than 2%, a program of aggressive management, i.e., early identification and isolation of suspected birds, resulted in reducing the mortality and incidence of prolapse to less than 0.1% within three weeks. This was in combination of treating more severe cases with physiological doses of estrogen. (All eggs from birds receiving estrogen were destroyed.)

B-6 Effect of debeaking

The effect of early and efficient cropping of the beak on the incidence of prolapse was of marginal benefit (Table 5).

B-7 Effect of bacteriological agents

Infecting hens with a bacteria isolated from the cloaca and vagina of the birds used in our experiments, Pasterella hemolytica, did not cause prolapse. Previously, Turner et al, 1979 (unpublished observations) isolated Nesseria pasterella and Micrococcus from prolapsed hens but these too were ineffective in evoking prolapse.

C. Biochemical Correlates of Oviductal Prolapse

1. Introduction

The endocrinology of the reproductive cycle in birds has not yet been fully elucidated (see vanTienhoven, 1981 for review). Apparently the signal for release of the ovulatory hormone is progesterone produced by the developing follicles. A role for testosterone has also been suggested. The role of estrogen in the ovulatory cycle of the hen is not known. Neither passive immunization (Johnson and vanTienhoven, pers. comm.) or active immunization against estrogen (Ailenberg and Shemesh, unpublished obs.) interferes with the ovulatory cycle. However prolonged administration with either tamoxifen (Wilson and Cunningham, 1981) or clomiphene (Shore and Shemesh, unpublished obs.) can cause a delay in the ovulatory peak. Nevertheless, it is clear that even at plasma concentrations below the sensitivity of our assay (<50 pg/ml), the hen can continue to have a normal rate of lay. Injections of pharmacological doses of estrogen (sufficient to cause marked hyperlipidemia) to healthy layers does not interfere with the normal cycle and may even increase the rate of lay.

The ovary in the hen consists of a number of large follicles which will ovulate during a cycle. The largest of the follicles (the primary follicle, F₁)

will ovulate first followed by the next largest follicles in size known as the secondary (F_2) and tertiary (F_3) follicles. It is the tertiary follicle which is responsible for the largest amount of steroid production and which is most responsive to LH (Huang et al, 1979). After ovulation, the post-ovulatory follicle (POF) continues to produce steroids and prostaglandins. This prostaglandin production reaches a peak about 25 hours after ovulation at the time of oviposition. In addition, the uterus itself can produce prostaglandin from arachidonic acid (Claeys et al, 1981). The relative importance of these two sources is not known but if the prostaglandin peak is blocked then oviposition does not occur. However this inhibition can be overcome and with 7 min. of a prostaglandin injection the inhibited hen will lay (Hertelendy and Fiellier, 1978). Two prostaglandins have been implicated (1) PGF which is responsible for contraction of the oviduct and (2) PGE which causes relaxation of the vagina.

In a series of preliminary experiments (Bendheim et al., 1980; Shemesh et al., 1981) we found that there was a high correlation between the incidence of prolapse and the intensity of the ambient lighting. Furthermore the concentrations of 17β estradiol were significantly lower in the blood of the prolapsed hen compared to the normal layer. In the present experiments, we therefore attempted to define more fully the endocrine status of the prolapsed hen by comparing them to normal hens as to (1) the hormonal profile under varying conditions; (2) the steroidogenetic capacity of the granulosa and theca cells of the ovary and (3) prostaglandin production in the oviduct and the post-ovulatory follicle.

C-2 Materials and Methods

Animals

Normal and prolapsed hens (8-10 mo. old) from commercial strains used in Israel were obtained from flocks with high incidence of prolapse mortality rate of from 2-12% a month. The hens were crosses between white Leghorns and Rhode Island reds. The hormonal profile of these birds in the time before and after oviposition is shown in Fig. 1. Clutches were highly variable ranging from 4-20 eggs per clutch. The mean inter-oviposition time was $25 \pm .3$ hours ($n=33$). The hens were housed in individual cages at ambient temperature with a dark:light cycle of 4:20 unless otherwise noted.

Chemicals

The cold steroids, arachidonic acid, 8Br-cAMP and collagenase were purchased from Sigma Chemical Co. (St. Louis, MO) and the labeled steroids from New England Nuclear (Boston, MA). Antibodies were raised in our laboratory. The labeled ($1\text{-}^{14}\text{C}$) arachidonic was purchased from Amersham, Buckinghamshire. The $\text{PGF}_{2\alpha}$, PGE_2 and thromboxane B_2 were a gift of the Upjohn Co., Kalamazoo, MI. The incubation medium for the cell preparations was Medium 199 with Earle's salts (Kimron Veterinary Institute, Beit Dagan, Israel) and 25 mM HEPES buffer (GBCO, Grand Island, NY, pH 7.4, supplemented with 5% calf serum and genticyclin (50 ug/ml; Sigma). The medium contained undetectable amounts of each of the steroids being measured (<50 pg/ml) before and after incubation without tissue. The bLH used in this study was bLH-NIH-B10 (1 $\mu\text{g/ml}$).

Preparation of cells and incubation

The follicles for incubation were taken from hens sacrificed 2-4 h prior to ovulation since this was the time shown by Huang and Nalbandov (1979) to be the time of maximal steroidogenesis. The theca and granulosa cell layers were separated and subjected to collagenase as described by them (Huang and Nalbandov, 1979). Cells were incubated in 1 ml of medium for 5 or 24 h under an atmosphere of 95% air and 5% CO_2 at 37 C. 3-5 incubations were done on cells from each follicle depending on the number of cells available. In one set of experiments, 70 ng of testosterone was added to the preparation to evaluate the aromatase enzyme activity since preliminary observations indicated that 50 ng was a maximal dose. The data are expressed as pg steroid produced per 5×10^5 cells over the time incubated.

Steroid assays

For determination of plasma or incubation medium plus cells steroid content, samples were extracted with ethyl ether (10 vol), the ether evaporated and the residue redissolved to the original volume with PBS. 100 μl aliquots were used for the radioimmunoassay determinations as previously described (Shemesh et al., 1978). Tracer amounts of labeled progesterone and estrogen were added prior to extraction and the samples were corrected for recoveries. Not all steroids were determined in each sample due to the insufficient volume. Values of less than 50 pg/ml in plasma or less than 50 pg/ 5×10^5 cells were considered non-detectable.

All values are mean $\pm$ S. E. M. and the significant differences were determined by Student's t test.

Measurement of prostaglandin activity

Hens were sacrificed 1-2 h prior to oviposition and the oviduct removed. The oviduct was scraped clean of mucosa and homogenized in 0.1 M potassium phosphate buffer, pH 8.1 (1.5 ml buffer to 1 g of wet tissue) with a Sorval Omni-mixer. The crude homogenate was centrifuged at 800 g and the supernatant recentrifuged at 108,000 g for 1 h. The resulting precipitate was designated as microsomes and resuspended to the original volume in 0.1 M potassium phosphate buffer, pH 8.1. Synthetase activity in the microsomal fraction was determined by incubating 1 ml aliquots of the suspension with 0.2 ml additional buffer containing 10 μ g arachidonic acid and 200 μ Ci of labeled arachidonic acid at 37 C for 30 min in open tubes with shaking. Incubations were terminated by the addition of 2M citric acid (0.25 ml) as described by Williams and Downing (5). The products of incubation were characterized by extracting twice with 10 vol of ether and separation on thin layer plated run in chloroform:methanol:acetic acid: water (90:9:1:0.65). Product formation (substrate converted) was taken as the radioactivity recovered from the plate after subtraction of boiled control values. In this study we did not follow those metabolites with a R_f close to that of arachidonic acid.

C-3 Results

Plasma steroid concentrations in normal and prolapsed hens

Table 6 shows the plasma 17β -estradiol and progesterone concentrations in hens from several commercial flocks which had a high incidence of prolapse. The samples were collected in field studies under varying conditions and were not related to the time of oviposition. There was significantly lower 17β -estradiol and progesterone plasma concentrations in birds with prolapse than in the healthy birds. In contrast, recovered hens were not significantly different from the normals.

Table 7 shows a controlled experiment where the same birds were sampled in prolapse and one month later when they no longer showed signs of prolapse. These birds were housed in individual cages at 50 lux and ambient temperature. Samples were taken within 1 h of oviposition. The 10 birds which recovered initially had undetectable (<50 pg/ml) concentration of 17β -estradiol while one month later the values were in the normal range. There was no significant difference in progesterone or testosterone concentrations at the start or end of the experiment. The difference in progesterone concentration between the field (Table 6) and the controlled study is most probably due to the more careful relating of the time of sampling to the time of oviposition.

17 β -estradiol and progesterone production by theca cells incubated for 5h or 24 h

Table 8 shows the production of progesterone and 17 β -estradiol by theca cells from the hierarchy of preovulatory follicles in the sequence in which they will ovulate, namely the first (F₁), second (F₂) and third preovulatory follicle (F₃). The follicle remaining after ovulation is termed the post-ovulatory follicle (POF). The principal steroid produced by the theca was 17 β -estradiol and its production was highest in the second and third preovulatory follicle. The amount of progesterone produced was small, especially after short term incubation, but it was readily detectable following 24 h of incubation.

17 β -estradiol production by thecal cells from normal and prolapsed hens in the absence or presence of 70 ng/ml of testosterone

In this experiment, theca cells from healthy hens were compared with theca cells from prolapsed hens (Table 9). Estrogen production was significantly lower in the prolapsed hen's theca especially considering the second and third preovulatory follicles ($p < 0.001$). Addition of testosterone (70 pg/ml) slightly increased the production of 17 β -estradiol by the normal hen's theca (25%) but evoked a 90% increase in estrogen production by the prolapsed hen's thecal cells. This augmented production of the prolapsed hen's 17 β -estradiol was about 50% of that observed with healthy hens. There was no significant difference in POF theca production of estradiol between the two groups.

17 β -estradiol and progesterone production by granulosa cells incubated for 5h or 24 h

The principal hormone produced by the granulosa cells was progesterone (Table 10). Very small amounts of estradiol were measured following 5 h of incubation and increased after 24 h of incubation. The amount of progesterone produced by the granulosa cells after 5 h of incubation was not appreciably elevated after 24 h of incubation.

Progesterone production by granulosa cells from healthy and prolapsed birds

The production of progesterone by granulosa cells obtained from healthy and prolapsed birds is shown in Table 11. There was somewhat lower progesterone in the prolapsed hen's granulosa from the second preovulatory follicle, but no differences were observed in the first and third preovulatory

follicles. The POF granulosa of prolapsed birds produced only half the amount of progesterone observed in the normal.

Effect of addition of 3mM-cAMP

The effect of the addition of 3mM Br-cAMP to incubations of granulosa and thecal cells from normal hens was studied in 5 h incubations. It was found that Br-cAMP increased the production of progesterone by the granulosa cells in all follicles studied but the increase was significant only for the secondary, tertiary and post-ovulatory follicle. The increase invoked by Br-cAMP in the theca cells was not significant but again the primary follicle was least responsive (Table 12).

Steroidogenesis by follicles from hens which had recovered from prolapse

Progesterone synthesis by the granulosa and estrogen synthesis by the theca was studied in follicles from birds 1-2 months after they had recovered from prolapse. The total progesterone produced by the three pre-ovulatory follicles did not differ from the normal. The most significant rise compared to prolapsed hen follicles was in the POF. Estrogen production by the theca cells from the preovulatory follicles did not return to normal values but were the same as found in the prolapse. The POF of the recovered hen produced significantly more estradiol than the normal or prolapsed theca.

Effect of LH on production of testosterone by the theca from the third follicle

Tertiary follicles were obtained from two normal and one prolapsed at the time of oviposition. To three of the six incubations per follicle, 1pg of bLH was added. (Preliminary investigations indicated that there was no effect of LH at 250 or 500 ng/ml.) It was found that progesterone production was increased by LH in all three birds from $575 \text{ pg}/5 \times 10^5$ theca cells to 3350 pg after 24 h of incubation. In contrast, while testosterone production from the two normal birds rose from 150 and 260 pg to 776 and 1010 pg/ 5×10^5 theca cells in the presence of LH, the prolapsed birds theca cells produced the same amount of testosterone in the presence or absence of LH (168 and 147 pg respectively).

Uterine prostaglandin synthetase activity in healthy, prolapsed and recovered hens

The prostaglandin metabolites produced by the uterine microsomes are shown in Fig. 2. Three distinct peaks were seen which co-migrated with PGF_2 , thromboxane B_2 , and PGE_2 respectively. It was found that the microsomes obtained from the prolapsed hen produced significantly less prostaglandin metabolites per mg protein than normal birds (Fig. 3). On the other hand, hens which had been prolapsed and subsequently recovered had values which were not significantly different from normal hens. Addition of estradiol in vitro in various concentrations indicated that at physiological concentrations estrogen stimulated prostaglandin synthetase activity in the uterus and in pharmacological ranges inhibited production (Fig. 4).

Prostaglandin production by the post-ovulatory follicle

The production of prostaglandin (PGE) by the POF near the time of oviposition is primarily by the theca rather than the granulosa (Table 14). The theca from the POF of prolapsed birds produced only 35% of the amount of PGE produced by that of normal hens (We could not validate the assay of PGF in this system).

In three birds which were injected with 100 μg of estrogen three times a week for three weeks, the POF thecal cells produced only 30% of the PGE produced by the normal hens theca.

C-4 Discussion

The present study demonstrates that hens with prolapsed oviduct have lower plasma estradiol-17 β and significantly lower production of estradiol-17 β by its theca cells. This is the first direct evidence this syndrome is related to the endocrine status of the hen although the work of Burmeister (1948) suggested the possibility some thirty years ago.

The results with the incubation of theca and granulosa cells are similar in part to that of Huang, Kao and Nalbandov (1979). We agree that the theca of the second and third preovulatory follicles are the principal source of estrogen in the ovarian follicle and that the theca is responsive to exogenous testosterone. It also agreed that the granulosa cells produce larger amounts of progesterone than the theca cells especially after short term incubation. On the other hand we did detect some 17 β -estradiol in the granulosa cell preparations. This observation is however in agreement with that of Wang (1982) who showed that both the theca and granulosa cells are capable of estradiol secretion. We also did not observe the hierarchy of progesterone secretion by the follicular granulosa cells reported by Huang et al., (1979) but this may be due to their use of hens with 4-5 egg cycles while our hens were a commercial breed with cycles of 4-10 eggs per clutch.

The lower synthetic capacity of the theca from the prolapsed hen is apparently due to defect in the metabolism of progesterone to testosterone (perhaps as the level of the 17, 20 lyase). The evidence for such a defect is indicated by (1) the observation that the theca cells from the prolapsed birds can synthesize estradiol-17 β from testosterone and the granulosa cells from the prolapsed birds did not show a serious defect.

in progesterone synthesis, and (2) the prolapsed bird responded to LH by an increase in progesterone but failed to increase testosterone production.

As to why the theca from the prolapsed birds was more responsive to exogenous testosterone than the healthy hen, this apparently due to sufficient testosterone being present in the hens theca prior to incubation to saturate the enzyme capacity.

It is generally agreed that the prostaglandins have a physiological role in oviposition in the hen (Hertelendy, Biellier and Todd, 1975; Hertelendy and Biellier, 1978; Day and Nalbandov, 1977; Wechsung and Houvenaghel, 1980). However, the source of the prostaglandin is not clear and two sites have been proposed: (a) the post-ovulatory follicle which increases its production of prostaglandins dramatically towards oviposition (Hertelendy and Biellier, 1978; Day and Nalbandov, 1977) and (b) the production of prostaglandin within the uterus itself from arachidonic acid (Wechsung and Houvenaghel, 1980). It has been shown that arachidonic acid can cause oviposition in the hen and that indomethacin will block the response to arachidonic acid (Wechsung and Houvenaghel, 1980). Furthermore, it has already been reported that the hen oviduct can synthesize prostaglandin metabolites from arachidonic acid and the principal metabolites are PGF_2 , thromboxane B_2 and PGE_2 (Claeys et al., 1981). This is similar to that found in our study of the normal hen oviduct. The novel finding in our experiments is that hens with oviductal prolapse had lower prostaglandin synthetase activity. This indicates that the oviductal production of prostaglandin does indeed have a physiological role in oviposition since the prolapsed hens have poor uterine contractility as evidenced by prolonged oviposition time. This of course, does not exclude in any way that there may also be a suppression of prostaglandin synthetase in the POF and indeed preliminary evidence in our laboratory indicates that this is so.

As to the factor responsible for the low prostaglandin synthetase activity in the prolapsed hen oviduct, it may be the result of the low plasma estrogen. Exogenous estrogen does increase the response of the oviduct to prostaglandin in ovariectomized hens (Rzasa & Ewy, 1976) and estrogen has been shown to increase prostaglandin synthetase activity in uterine homogenates from ovariectomized guinea pigs (Naylor & Poyser, 1975). Furthermore, our present data indicate that some concentrations of estradiol do significantly increase prostaglandin synthetase activity by the hen uterine microsomal fraction. It is therefore possible that the low plasma estradiol plays a direct role in the low prostaglandin synthetase activity observed in the prolapsed hen.

The post-ovulatory follicle is also affected in the prolapsed hen. This is indicated by a number of lines of evidence: (1) PGE production by theca cells is only 35% of comparable healthy hens; (2) in recovered birds only the POF showed a return of progesterone and estradiol production to a level comparable to normals; (3) injection of pharmacological doses of estrogens into healthy birds had much lower PGE production than normals but in the range seen in prolapsed birds. It therefore seems likely that the reduced prostaglandin synthetase activity seen in the oviduct is also characteristic of the POF of the prolapsed bird. Since prostaglandin production by the POF plays a critical role in oviposition (Hertelendy and Biellier, 1978), a defect in the POF could be the site of the most serious defect in biochemistry of the prolapsed bird.

As to the environmental factors responsible for prolapse, no definite conclusions can be drawn as we were unable to cause prolapse in healthy birds either by estrogen, testosterone, or anti-estrogen injections.

However, we were able to evoke prolapse in birds with a previous history of prolapse using pharmacological doses of estrogen and both pharmacological doses and anti-estrogen prevented the prolapse hen from recovering. Furthermore, injection of pharmacological doses of estrogen in healthy hens produced the same biochemical defects as seen in the prolapsed hens. It would therefore seem probable that estrogenic substances in the feed (which have both anti-estrogenic and estrogenic properties) aggravated by non-specific stress (crowding, intense light, high rate of lay) have a central role in the evolution of prolapse. Evidence for this derives from the observation that we have detected estrogenic activity in 12 different components of Israeli animal feed, including soybean meal, sorghum and corn which are the principal components of chicken feed. Furthermore, estrogenic substances in feed have already been implicated in prolapsed uterus in birds (Meronuck et al., 1970), sheep (Bennetts, Underwood, Shier, 1946) and sows (Bristol, Djurickovi, 1971).

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D. Evaluation of the research achievement with respect to the original proposal

Our original proposal had five objectives:

1. To define the hormonal imbalance characteristic of hens with prolapse.
2. To elucidate the endocrine control mechanism of oviductal prolapse by in vitro studies of follicles and uterus with emphasis on prostaglandin synthesis by the post-ovulatory follicle and the uterus which control oviposition.
3. To investigate the effect of different photoperiods on the appearance of the syndrome.
4. To develop a hormonal treatment for prolapsed birds.
5. To develop a program of prevention by altering the environmental factors which acerbate the tendency to prolapse.

All of these objectives were accomplished. There of course remains a great deal to be learned about the endocrinology of prolapsed hens. However, such efforts depend on developments in the field of avian endocrinology which is a comparatively new field and much of the information which is well established in mammals is not available for the chicken. As to the treatment of the syndrome, we did find an effective treatment for hens in batteries. However, we have no solution for hens in trap nests where identification and isolation of prolapsed hens is not practical.

E. Conclusions

1. Prolapsed oviduct in the hen is a treatable syndrome. It is aggravated by non-specific stress such as crowding and intense light. No correlation could be demonstrated between incidence of prolapse with bacteriological, viral or genetic factors.

2. Estradiol is biphasic in the prolapsed hen. In physiological doses, it evokes rapid recovery and in pharmacological doses it prevents recovery.
3. Early identification and isolation of suspected birds can reduce mortality from prolapse from 2% to less than 0.1% within three weeks in hens housed in batteries.
4. Low or undetectable plasma estradiol concentrations are characteristic of the prolapsed bird. This is unrelated to strain, rate of lay or time of sampling. It was found that when the bird recovered clinically, estradiol concentrations returned to normal.
5. The source of the low estradiol concentration is low estradiol production by the theca of pre-ovulatory follicles. This biochemical defect is due to low conversion of progesterone to testosterone.
6. Prostaglandin production in the oviduct and post-ovulatory follicle of the prolapsed bird is deficient. This is related to the concentration of estradiol with physiological concentrations increasing and pharmacological dose inhibiting prostaglandin synthetase activity.
7. Low prostaglandin concentrations interfere with proper contraction of the oviduct and relaxation of the vagina at oviposition. The laying hen therefore increases its pressure at oviposition which results in the prolapse.

F. Description and results of cooperation

Since Dr. Shemesh and Dr. Shore were new to the field of avian endocrinology, the advice and information provided by Dr. vanTienhoven was extremely helpful in formulating our experiments. Co-operation with actual measurements of samples was not feasible due to restrictions in import of animal samples to the U. S. Both Drs. Shemesh and Shore visited Dr. vanTienhoven in the course of the project for consultation.

G. Thesis (hypothesis)

Prolapsed oviduct in the hen is caused by a lack of estradiol production by the ovary. This estradiol is essential for the production of prostaglandins needed for oviposition. In the absence of adequate uterine contractility, the hen expels the egg by increased abdominal pressure which results in the prolapse. Replacing the estrogen lack by injections of estrogen therefore results in rapid recovery by returning prostaglandin synthesis to its proper level.

H. Benefit to agriculture

The general incidence of prolapsed oviduct in hens is not known so the overall benefit to agriculture can be calculated. In the four farms from different parts of Israel we studied in 1982 over 8000 hens died from prolapse. This represented an overall mortality from prolapse of from 10 to 20% per coop. The data presented in this report indicates that at a very low cost (treatment cost less than 1 cent per bird) the overall mortality could be reduced to 1% per coop.

I. Inventions

The use of estradiol for curing prolapse in hens would represent a new use for an established drug. Our understanding is that this is not patentable. However, finding the most effective and safest form of estradiol for treating the bird and producing it packaged for this purpose could have commercial implications.

J. Publications

Chapter in books

1. Bendheim, U., Shemesh, M., Totach, A. and Lavi, S. (1980).

Veränderungen der Ovarialhormone bei Eileiterverfall in Legehennen.

In: Proceedings of the European Poultry Conference, World's

Poultry Conf. Assn. German Branch (Fed) Vol. IV: pp. 286-292.

2. Shemesh, M., Shore, L.S., Bendheim, U., Lavi, S. and Weismann, Y. (1982)

Correlation between estrogen concentrations, light and prolapsed uterus

in laying hens. In: Advances in Pathology (E.Levy, ed.) Pergamon Press:

Oxford Vol. I: 273-276.

Abstracts

1. Shemesh, M., Shore, L.S., Bendheim, U., Lavi, S. and Weismann, Y. (1981)

Comparative study of estrogen biosynthesis by ovarian theca cells from normal and prolapsed layers. Poultry Sci. 60:1731.

2. Bendheim, U., Shemesh, M., Totach, A., Shore, L. and Perl, S. (1981)

Relationship between hormonal profile and clinical signs in laying hens with prolapsed cloaca and oviduct. Refuah Vet. 38:50-51.

Table 1

Observations on a 7 months old flock afflicted with oviductal prolapse
(pathiognomic parameters), Beit Meir, 1978

	<u>Lit</u>	<u>Shaded</u>
No. of Birds	1300	1440
No. prolapsed	15	5
Number dead 12 days later	12	4
Cloacitis	21	0
Number of eggs	1220	900
Percent layers	93.8	62.5
Blood stained	70 (5.4%)	9 (1%)

Table 2

Effect of reducing light on prolapsed oviduct in layers, Maagan 1982

	<u>Lit half</u>	<u>Shaded half</u>
Mortality rate	4.6%	4.4% (before shading)
1st month	3.2%	2.9%
2nd month	1.5%	1.7%

Table 3

Effect of illumination on mortality rates from oviductal prolapse,
Beit Meir, 1982

	<u>Lit</u>	<u>Shaded</u>
Monthly mortality rate	4.3%	3.3%
Rate of lay	79.1%	70.3%

Table 4

Effect of reducing photoperiod on hens recovering from prolapse,
Beit Dagan, 1981, (8-10 birds in each group)

Photoperiod (light: dark)	Time to disappearance of prolapse (days $\pm$ s.e.m.)	% lay
6:18	20 $\pm$ 4	33
8:16	18 $\pm$ 0.5	55
16:8	29 $\pm$ 2	62
18:6	27 $\pm$ 3	60

Table 5

Effect of debeaking on 2730 layers, Beit Meir, 1982

	Before debeaking	After debeaking
Mortality rate/week	15	6
Rate of lay	79%	66%
Blood stained eggs	166	140

Table 6

Plasma steroid concentrations in healthy, recovered and prolapsed birds in samples obtained in field studies of hens having a high incidence of prolapse.

Type	Estradiol-17 β (pg/ml)	Progesterone (pg/ml)
Healthy	475 $\pm$ 45 (64)	874 $\pm$ 113 (22)
Recovered	374 $\pm$ 40 (32) ^c	1126 $\pm$ 107 (26) ^c
Prolapsed	60 $\pm$ 12 (47) ^a	510 $\pm$ 81 (33) ^b

Each value is the mean $\pm$ s.e.m. of results obtained from individual hens; the number of hens is shown in parentheses; ^aP<0.001 vrs healthy birds; ^bP<0.01 vrs healthy birds; ^cNS vrs healthy birds.

Table 7

Plasma steroid concentrations in birds which were prolapsed and subsequently recovered. The same bird was sampled in prolapse and 1 month later. Samples were drawn within 1 h of oviposition.

Type	Estradiol 17 β (pg/ml)	Progesterone (pg/ml)	Testosterone (pg/ml)
Prolapsed	< 50 (15)	1043 $\pm$ 105 (9)	227 $\pm$ 68 (13)
Recovered	434 $\pm$ 55 (10)	970 $\pm$ 95 (8)	278 $\pm$ 69 (7)
Did not recover	< 50 (5)	908 $\pm$ 268 (4)	117 $\pm$ 94 (4)

Each value is the mean $\pm$ s.e.m. of results obtained from individual hens; the number of hens is shown in parentheses.

Table 8

Steroid production by theca cells from normal layers following 5 or 24 h of incubation in supplemented medium 199.

Follicle		Progesterone (pg/5x10 ⁵ cells)	Estradiol 17 β (pg/5x10 ⁵ cells)
F ₁	5h	ND(9)	94 $\pm$ 13 (9)
	24h	1084 $\pm$ 280 (20) ^a	116 $\pm$ 13 (24)
F ₂	5h	ND(9)	124 $\pm$ 18 (9)
	24h	688 $\pm$ 130 (20) ^a	599 $\pm$ 98 (24) ^a
F ₃	5h	82 $\pm$ 50 (9)	354 $\pm$ 60 (24)
	24h	1150 $\pm$ 150 (20)	925 $\pm$ 141 (24) ^a
POF	5h	250 $\pm$ 130 (9)	85 $\pm$ 20 (9)
	24h	256 $\pm$ 97 (18)	87 $\pm$ 10 (24)

Each value is the mean $\pm$ s.e.m. of the results obtained from separate incubations; the number of incubations is shown in parentheses.

^a P < 0.001; 5h vrs. 24 h incubation.

Table 9

Estradiol-17 β synthesis (pg/5x10⁵ cells) by theca cells following 24 h incubation in supplemented medium 199 in the presence or absence of 70 ng testosterone

Follicle	Normal		Prolapsed		Normal+testosterone		Prolapsed + testosterone	
F ₁	116 $\pm$ 13 (24) ^{ab}		85 $\pm$ 14 (27) ^b		135 $\pm$ 23 (17) ^a		133 $\pm$ 27 (19) ^{ab}	
F ₂	599 $\pm$ 98 (24) ^a		154 $\pm$ 22 (27) ^b		530 $\pm$ 75 (17) ^a		262 $\pm$ 19 (19) ^c	
F ₃	935 $\pm$ 141 (24) ^a		217 $\pm$ 25 (27) ^b		1405 $\pm$ 146 (19) ^a		475 $\pm$ 69 (19) ^b	
POF	87 $\pm$ 10 (24) ^a		74 $\pm$ 5 (27) ^a		170 $\pm$ 42 (24) ^b		198 $\pm$ 62 (19) ^b	

Each value is the mean $\pm$ s.e.m. of results obtained from separate incubations; the number of incubations is shown in parentheses;

^{abc} Estimations with different letters in the same line are significantly different from each other (P < 0.005).

Table 10

Steroid production by granulosa cells from normal layers following 5 or 24 h incubation in supplemented medium 199.

Follicle		Progesterone (pg/5x10 ⁵ cells)	Estradiol-17 β (pg/5x10 ⁵ cells)
F ₁	5h	7500 $\pm$ 540 (6)	97 $\pm$ 8 (9)
	24h	5160 $\pm$ 150 (21) ^a	212 $\pm$ 29 (12) ^a
F ₂	5h	5360 $\pm$ 1036 (6)	77 $\pm$ 7 (9)
	24h	8850 $\pm$ 580 (21) ^a	209 $\pm$ 29 (12) ^a
F ₃	5h	5208 $\pm$ 408 (6)	54 $\pm$ 7 (9)
	24h	5060 $\pm$ 580 (21)	155 $\pm$ 41 (12) ^a
POF	5h	5910 $\pm$ 408 (6)	96 $\pm$ 10 (9)
	24h	5680 $\pm$ 580 (21)	198 $\pm$ 20 (12) ^a

Each value is the mean $\pm$ s.e.m. of results obtained from separate incubations; the number of incubations is shown in parentheses. ^aP<0.001; 5 h vrs 24h.

Table 11

Progesterone synthesis (pg/5x10⁵ cells) by avian granulosa cells from hens with prolapse and healthy hens. Cells were incubated for 24h in supplemented tissue culture medium 199.

Follicle	Normal layers	Prolapsed layers	P
F ₁	5160 $\pm$ 150 (21)	4949 $\pm$ 250 (26)	NS
F ₂	8850 $\pm$ 460 (21)	5190 $\pm$ 580 (19)	<0.001
F ₃	5060 $\pm$ 580 (21)	5230 $\pm$ 400 (19)	NS
POF	5860 $\pm$ 710 (21)	2820 $\pm$ 570 (26)	<0.001

Table 12

Effect of 8 BrCAMP on granulosa and theca cell steroidogenesis. (5 h incubation)

Follicle	Progesterone	%inc ^a	Estradiol-17	%inc ^b
F ₁	8160 $\pm$ 830 (6)	9%	99 $\pm$ 14 (9)	6%
F ₂	7191 $\pm$ 840 (6)	34%*	177 $\pm$ 28 (9)	37%
F ₃	6076 $\pm$ 400 (6)	28%*	412 $\pm$ 80 (9)	16%
POF	6660 $\pm$ 400 (6)	13%*	111 $\pm$ 16 (9)	40%

^acompared to Table 10

^bcompared to Table 8

*P<0.005

Table 13

Steroid production by follicles from hens which had recovered from prolapse

Follicle	Progesterone	%change ^a	Estradiol-17	%change
F ₁	7016±590 (30)	42%*	85±6 (30)	0
F ₂	5942±950 (30)	14%	139±14 (35)	-11%
F ₃	6076±338 (30)	14%	315±32 (12)	+45%
POF	4913±660 (23)	74%*	181±12 (35)	144%*

^a compared to prolapsed follicles; * P<0.001

Table 14

PGE production by the post-ovulatory follicle at the time of oviposition
(ng PGE/2x10⁴ cells/24 h)

	Granulosa	Theca
Normal	0.97±.34 (17)	2.38±.30 (17)
Prolapsed	0.10±0.03 (14)	0.82±.10 (14)
Normal + E ₂	0.13±0.04 (9)	0.74±.14 (9)

Fig. 1

Fig.2

Fig. 3

Fig.4 Effect of estradiol on the conversion of arachidonic acid to prostaglandin metabolites by uterine microsomes.