AN ABSTRACT OF THE THESIS OF

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Title: EFFECT OF POSTERIOR PITUITARY HORMONES ON FERTILITY IN THE DOMESTIC FOWL

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An attempt was made to create a hormonal environment of the uterus comparable to that near the time of natural laying and to eliminate the effect of a hard-shelled egg on fertilization. Hens without hard-shelled eggs in their uteri were injected with the posterior pituitary hormones, oxytocin and arginine vasopressin, at various times before or after artificial insemination. Subsequent fertility was reduced by the hormone injections but not to the extent observed when oviposition occurred naturally at various time intervals before or after insemination. Fertility was reduced significantly when 3 or 5 I. U. of oxytocin was injected immediately before insemination, only slightly when injected 30 minutes before and not at all when injected one, two or five hours before insemination. Fertility was reduced significantly when 5 I. U. of oxytocin was injected immediately or 30 minutes after insemination and slightly reduced when injected one or

two hours after insemination. Also there was a tendency for fertility to be reduced when 3 I. U. of arginine vasopressin was injected immediately, 30 minutes or one hour after insemination but the reductions were not statistically significant. A combination of 2.5 I. U. of oxytocin and 1.5 I. U. of vasopressin injected immediately after insemination caused a greater reduction in fertility than 3 I. U. of vasopressin.

A single intravenous injection of 5 I. U. of oxytocin one or two hours after insemination caused as much reduction in fertility as an intravenous injection of 3 I. U. preceded by two or three subcutaneous injections of 1 I. U. of oxytocin.

Diluting semen with 2 I. U. of oxytocin per ml or with 0. 125 mg of epinephrine per ml tended to reduce fertility but not significantly so.

Subcutaneous injection of hens with 1 mg of epinephrine 20 minutes before insemination tended to increase fertility but did not counteract the adverse effects of oxytocin. Also intravenous injections of 0. 5 mg of epinephrine or the addition of 0. 125 mg of epinephrine per ml of semen did not overcome the reduction in fertility related to oviposition shortly after the time of insemination. Egg production was significantly reduced by subcutaneous injections of epinephrine but was not affected by intravenous injections of epinephrine.

Intravenous injection of male chickens with 0.05 to 5 I. U. of oxytocin shortly before manual ejaculation tended to decrease semen volume, and increase concentration of sperm but did not significantly affect the total number of sperm per ejaculate. Oxytocin injections had no effect on the fertilizing capacity of the semen or the hatchability of the fertile eggs.

Effect of Posterior Pituitary Hormones on Fertility in the Domestic Fowl

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I. INTRODUCTION

In chickens morning inseminations have been shown to fertilize a smaller percentage of eggs than afternoon inseminations. This finding gave rise to the hypothesis that the presence of a hard-shelled egg in the uterus at the time of insemination has an adverse effect on fertilization in the domestic hen. The results of further investigations supported this hypothesis but also demonstrated that this adverse effect occurs only if insemination is done within the last four hours prior to oviposition.

It was later shown that inseminations made within two hours after oviposition also resulted in a low fertilization rate. Since the uterus would not have contained an egg at this time it was concluded that some factor other than the physical presence of an egg is involved in the reduced fertility following inseminations near the time of oviposition.

Hormones released from the posterior pituitary gland initiate oviposition and their concentration in the blood is increased shortly before and after oviposition. Hens given intravenous injections of oxytocin or inseminated with semen diluted with oxytocin exhibit a reduction in fertility similar to that observed following insemination within a short time before or after oviposition.

This study was a further investigation of the effects of posterior pituitary hormones on fertility in the domestic fowl. The primary objective was to determine if the presence of these hormones or changes induced by them was the cause of reduced fertility following insemination shortly before or after oviposition.

The effect of oxytocin on semen quantity and quality from cockerels also was studied.

II. REVIEW OF LITERATURE

Fertilization in the Fowl

Spermatozoa are deposited in the vagina at the posterior end of the oviduct and then are transported to the infundibulum where fertilization of eggs occurs (Patterson, 1910; Olsen and Neher, 1948). The laying of the first fertile egg, or onset of fertility, may be as early as 19.5 hours after mating (Nicolaides, 1934). Since the average time required for egg formation is about 25 hours (Warren and Scott, 1935) and fertilization occurs soon after ovulation it is apparent that spermatozoa reach the infundibulum quite rapidly. Following a single mating a hen may continue to lay fertile eggs for as long as four weeks, however the average duration of fertility is about two weeks (Nicolaides, 1934).

Onset of fertility and duration of fertility are two of the factors which determine the success of artificial insemination. In addition the zygotes must be capable of normal development. Thus, any factor which has an adverse effect on spermatozoa transport and storage within the female reproductive tract may reduce the fertilization rate. Physical damage to the spermatozoa themselves can adversely affect fertilization rate and conceivably the normal subsequent development of the zygote. Fertilization by senescent sperm has been shown to

cause a high incidence of embryonic abnormalities (Dharmarajan, 1950; Olsen and Marsden, 1953).

Time of Insemination and its Effect on Fertility

The development of a practical technique of artificial insemination in the chicken by Burrows and Quinn (1939) was a significant contribution to the field of poultry breeding and research. Since the development of this technique questions have arisen as to how to obtain maximum fertility with its use. Answers to questions concerning the time of day or stage of egg formation when artificial insemination should be performed are still being sought. It has been demonstrated that natural matings restricted to the forenoon result in lower fertility than unrestricted matings or matings restricted to the afternoon (Gracewski and Scott, 1943; Parker, 1950). Similar results have been obtained with artificial inseminations which demonstrated that noon or late afternoon inseminations resulted in higher fertility than that obtained with morning inseminations (Parker, 1945; Bornstein et al., 1960; Parker and Arscott, 1965; Johnston, 1967).

The possibility exists that diurnal changes in semen quality influences the fertilization rate of hens artificially inseminated at various times of the day. If this was an important factor concentration of spermatozoa in the semen and motility of the spermatozoa might be expected to be lower in semen collected in the morning hours than in

semen collected in the afternoon. However both concentration and motility of the spermatozoa have been shown to be slightly higher in semen collected during morning hours than in that collected in the afternoon (Parker, McKenzie and Kempster, 1940). If the quality of semen collected during the morning is as high as that collected in the afternoon the cause of reduced fertility following morning inseminations is due to conditions within the female.

In the female the stage of egg formation is different in the morning than it is in the afternoon. Table 1 gives a brief description of egg formation in the chicken.[1] During the morning hours as high as 61% of the hens may have hard-shelled eggs in their uteri whereas in the afternoon the incidence of hard-shelled eggs may be as low as 9% (Warren and Scott, 1935; Warren and Gish, 1943). The presence of a hard-shelled egg in the oviduct at the time of insemination has been shown to cause a reduction in fertility when compared to inseminations at other stages of egg formation (Moore and Byerly, 1942; Malmstrom, 1943; Parker, 1945; Bornstein et al., 1960). The results of Bornstein et al. (1960) indicate that this reduction in fertility is primarily due to a high percentage of totally unfertilized hens. The percentage of fertile eggs laid by fertilized hens is not significantly affected. This substantiates the view that diurnal variations in semen quality are not

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[1] Tables 1 through 22 are presented in the Appendix.

responsible for the fertility differences observed between morning and afternoon inseminations. Their results also indicate that the presence of a hard-shelled egg in the oviduct is not always detrimental to fertilization. Further evidence for this is provided by the results of Parker and Arscott (1965). Inseminations at 9 p.m. when most of the hens had eggs in their uteri resulted in fertility comparable to that of hens inseminated at mid-afternoon. More recent research indicates that the presence of a hard-shelled egg in the uterus at the time of insemination has an adverse effect on fertilization only when that egg is laid within three to four hours after insemination (Johnston and Parker, 1970; Hughes, 1970).

The presence of a hard-shelled egg in the uterus at the time of insemination may interfere with sperm transport and storage within the female reproductive tract. These processes are vital to fertility in the chicken since fertilization occurs in the anterior end of the oviduct (Patterson, 1910; Olsen and Neher, 1948) and the sequential nature of ovulation in birds requires the life span of the spermatozoa to be prolonged and protected from being swept out by successive descending eggs (Lorenz, 1966).

Transport and Storage of Spermatozoa in the Female

In the chicken spermatozoa can reach the upper portion of the female reproductive tract within 30 minutes or less after their

deposition in the vagina (Mimura, 1939; Allen and Grigg, 1957; Boone, 1966; Donovan, Boone and Turk, 1969). However, an ovum in the magnum or lower portion of the oviduct has been reported to block sperm movement until the egg is laid, after which progression is rapid (Mimura, 1939). Rapid transport of spermatozoa in the female reproductive tract has also been demonstrated in mammals such as the rat (Blandau and Money, 1944), dog (Evans, 1933), ewe (Stark, 1949) and cow (VanDemark and Moeller, 1951).

Normal spermatozoa are rarely found in the lumen of the fowl's oviduct beyond 24 hours after mating (Warren and Kilpatrick, 1929). Walton and Whetham (1933) were unable to find sperms in the oviducts of females after mating, although the females laid fertile eggs up to 15 days after mating and fertility persisted after irrigation of the oviduct and peritoneal cavity with spermicidal solutions. Van Drimmelen (1945) found normal active spermatozoa in the infundibulum of the fowl's oviduct up to 14 days after insemination. He later found "sperm-nests" in crypts or gland ducts in the mucous lining of the funnel region of the oviduct after intraperitoneal inseminations and a few of these spermnests were observed after natural mating (Van Drimmelen, 1946). This finding offers an explanation of the failure to terminate fertility by irrigation of the oviduct with spermicidal solutions (Walton and Whetham, 1933).

Spermatozoa storage sites also have been described in the "uterovaginal glands" located at the junction of the uterus and vagina (Fujii and Tamura, 1963; Bobr, Lorenz and Ogasawara, 1964; Bobr, Ogasawara and Lorenz, 1964). The histological structure and ultrastructure of these glands are similar to those in the infundibulum (Schindler et al., 1967). Bobr, Lorenz and Ogasawara (1964) found spermatozoa in the infundibular glands only after special and unusual insemination techniques and concluded that the uterovaginal glands are the normal storage sites for spermatozoa rather than the infundibular glands. Spermnests have also been observed in the uterovaginal glands of the turkey but not in the infundibular glands (Verma and Cherms, 1964, 1965). The cells of the uterovaginal glands produce a secretion which is presumably unique in composition and may be beneficial to the spermatozoa (Gilbert, Reynolds and Lorenz, 1968a).

Sperm may be transported to the site of fertilization by sperm motility, ciliary action in the female reproductive tract, contractions of the female reproductive tract, or by a combination of these factors. Parker (1930, 1931) observed a narrow band of cilia in the oviduct of the pigeon which beat toward the anterior end. He suggested that sperm deposited in mating were transported through the vagina and uterine portion of the oviduct by antiperistaltic muscular action after which they were transported through the remainder of the oviduct by ciliary action. Mimura (1941) reported that copulation or digital

pressure applied in the course of artificial insemination induced antiperistalsis of the oviduct, relaxation of the sphincter between the vagina and uterus, and transport of oviducal contents. He concluded that neither sperm motility nor ciliary movement plays an important role in the transport of spermatozoa in the female reproductive tract.

Using the technique of labeling sperm with ^{32}P it has been shown that a very small percentage of live sperm progress past the uterus after intravaginal insemination and dead sperm inseminated in the same manner do not enter the upper part of the oviduct. But after intrauterine insemination both live and dead sperm reach the infundibulum in large numbers and with equal rapidity. Thus, the mechanism of sperm transport differs on either side of the uterovaginal junction (Allen and Grigg, 1957). These authors felt that the "bearing-down" reflex contractions of the lower part of the oviduct (Sykes, 1954, 1955a) may provide the mechanism of sperm transport in the oviduct anterior to the vagina and that the uterovaginal junction, like the cervix in mammals, constitutes a barrier to sperm passage (Braden, 1953; Braden and Austin, 1954; Dobrowolski and Hafez, 1970). The function of the uterovaginal junction as a "sperm barrier" had been suggested earlier when it was found that semen diluted with a Tyrode-glycerol solution fertilized no eggs with intravaginal inseminations but produced good fertility (73%) after intrauterine insemination (Allen and Bobr, 1955). The fact that three hens, which

were sterile with normal insemination techniques, exhibited high fertility (90%) after intraperitoneal insemination substantiates this view (Grigg and Skaller, 1958).

Inseminations above the uterovaginal junction with semen from normal or low-fecundity cocks results in increased early mortality and embryo abnormalities suggesting that this region may possess a sperm-selection mechanism (Ogasawara, Lorenz and Bobr, 1966; Van Krey, Ogasawara and Lorenz, 1966; Lorenz and Ogasawara, 1968). This is further indicated by the fact that turkey semen deposited anterior to the uterovaginal junction in chickens resulted in a higher number of spermatozoa in the upper portion of the oviduct and increased the percentage and duration of fertility in comparison to intravaginal insemination (Kempenich-Pinto et al., 1970; Schindler et al., 1970).

One function of the utero-tubal junction in mammals is to control the number of spermatozoa reaching the upper portion of the reproductive tract. The total number of spermatozoa allowed to reach the fertilization site is a compromise between insuring sufficient numbers for fertilization and preventing excessive numbers to avoid polyspermy (Austin and Braden, 1952). This same function may be applicable to the uterovaginal junction in the fowl's oviduct. Although polyspermy is a frequent occurrence in the avian species and apparently causes no damage to the embryo (Romanoff, 1960) the excessive mortality

following insemination above the uterovaginal junction was thought to be due to excessive polyspermy (Van Krey et al., 1966).

Posterior Pituitary Hormones and Sperm Transport

Activity of the female reproductive tract has been suggested as the primary means of spermatozoa transport within the female. Uterine contractions can be induced in the cow by a variety of stimuli including milking, massaging the vulva or cervix, the presence of a bull, noncopulatory mounting and copulation (VanDemark and Hays, 1951, 1952). It has been demonstrated that both injections of oxytocin and stimulation of the reproductive organs of the cow induces milk "let-down" (Ely and Petersen, 1941; Hays and VanDemark, 1953b). Blood taken after, but not before, stimulation of the reproductive organs caused contraction of perfused excised uteri, similar to that caused by oxytocin, indicating that stimulation of the reproductive organs of the cow causes the release of oxytocin (Hays and VanDemark, 1953a, b). In the excised, perfused genital tract of the cow addition of oxytocin to the perfusing fluid after sperm had been deposited in the cervix enhanced sperm transport in the tracts in both the follicular and the progestational phases of the estrous cycle (VanDemark and Hays, 1955). Thus, the release of oxytocin during mating may initiate the uterine contractions responsible for sperm transport in the female.

Pituitrin, a posterior pituitary extract, has been shown to cause contractions of the avian uterus (Morash and Gibbs, 1929; McKenney, Essex and Mann, 1932) and to induce premature laying in the pigeon (Riddle, 1921) and chicken (Burrows and Byerly, 1942; Burrows and Fraps, 1942).

Chauvet, Lenci and Acher (1960) reported that the neurohypophysis (posterior pituitary) of the hen contains oxytocin, arginine vasopressin and arginine vasotocin. Although arginine vasopressin has only a slight oxytocic effect in mammals its ability to cause contraction of the avian uterus is equal to or greater than that of oxytocin. Evidence has also been presented that arginine vasopressin is absent in the hen and that the chicken uterus is more sensitive to vasotocin than to either vasopressin or oxytocin (Munsick, Sawer and Van Dyke, 1960; Heller and Pickering, 1961).

The resting level of plasma arginine vasotocin in the hen is approximately 50 to 80 μU/ml (Douglas and Sturkie, 1964). Two to 20 minutes before oviposition this value is about 150 μU/ml and during oviposition the concentration is 30 to 150 times higher than the resting level (Douglas and Sturkie, 1964; Opel, 1966; Sturkie and Lin, 1966). After oviposition the concentration drops rapidly. A corresponding drop in vasotocin content of the posterior pituitary gland just prior to oviposition along with a slight decrease in oxytocin content has been observed (Tanaka and Nakajo, 1960, 1962).

The sensitivity of the oviduct to oxytocin, vasopressin and vasotocin increases toward the time of normal oviposition (Gilbert and Lake, 1963; Rzasa and Ewy, 1970).

It is possible that spermatozoa inseminated near the time of oviposition are affected in some way by the hormones which cause oviposition. Addition of oxytocin directly to cock semen or to a saline diluent decreases sperm motility in vitro (Hughes and Parker, 1970). That a positive correlation exists between motility of spermatozoa in cock semen and the resulting fertility following inseminations is well documented (Parker, McKenzie and Kempster, 1942; Shaffner and Andrews, 1948; Allen and Champion, 1955; Cooper and Rowell, 1958; McDaniel and Craig, 1962; Soller, Schindler and Bornstein, 1965).

The blood supply of the uterovaginal sperm-host glands is extensive and in close proximity to the gland cells. This may allow the spermatozoa to be influenced by hormones or other blood borne substances (Gilbert, Reynolds and Lorenz, 1968b).

Posterior Pituitary Hormones and Fertilization

As stated above stimulation of the female reproductive organs causes the release of oxytocin which initiates uterine contractions and aids in spermatozoa transport. This effect on spermatozoa transport could influence fertilization rate.

Ewes that were allowed to associate with vasectomised rams immediately after insemination showed an increase in fertility of 10% over ewes which were not allowed to associate with the rams (69.0% vs. 58.9%). It was postulated that the ram stimulus may have caused the release of an oxytocin-like substance which facilitated sperm transport thus raising the conception rate (Restall, 1961). However, blood extracts of ewes in natural estrus showed no increase in the concentration of oxytocin following stimulation by copulation (Hawker, Roberts and Walmsley, 1959).

Massaging the reproductive tract of cows for one to two minutes before insemination increased conception rate with 78% of 51 treated cows conceiving compared to 59% of 80 untreated cows (Tjupic, 1955).

Hays, VanDemark and Ormiston (1958) reported that intravenous injections of 15 I. U. of oxytocin within five minutes after natural mating resulted in a significant increase in the conception rate of one group of normal cows (controls 56% vs. oxytocin 84%). A similar trend (not statistically significant) was found in a second group of normal cows and in two groups of "hard-to-settle" cows. The same treatment in one group of artificially inseminated cows failed to increase conception rate (controls 73% vs. oxytocin 67%).

A similar experiment with gilts (Stratman, Self and Smith, 1959) showed that intravenous injections of oxytocin (10 I. U.) increased the percentage of fertilized ova (controls 58% vs. oxytocin

71.5%) and increased mean number of pigs per litter (controls 5.0 vs. 6.8). Oxytocin added to semen (10 I. U. /2.5 billion sperm) resulted in a nonsignificant reduction in the percentage of fertilized ova although a very slight increase in the mean litter size was observed.

In chickens, intramuscular injections of 3 I. U. of oxytocin immediately before artificial insemination resulted in a slight but nonsignificant increase in the percentage of fertile eggs laid two to four days following insemination (Hawes, 1962). Contrary to these results, Hughes and Parker (1970) found that intravenous injections of 5 I. U. of oxytocin before or after insemination caused significant reductions in fertility of eggs laid two to nine days following insemination. Also dilution of semen with oxytocin (2 I. U. /ml) significantly reduced fertility.

Biological actions of hormones involve the changing of the permeability of cell membranes or activation or inhibition of enzyme systems (Turner, 1966). Thus, the effects of oxytocin on fertility could be due to changes in the oviducal environment.

Inactivation of Hormones by Enzymes

Through the use of electrophoresis two enzymes were found in the plasma of pregnant women which were capable of inactivating oxytocin and vasopressin with considerable rapidity (Page et al., 1961). Oxytocinase, a cystine amino-peptidase (CAP), splits tyrosine

from that half of cystine bearing the terminal amino group, thus opening the pentapeptide ring portion of oxytocin to render it inactive. The activity of oxytocinase may be measured chemically by the use of a synthetic substrate, cystine di-beta naphthylamide. Page et al. concluded that oxytocinase is produced by the placenta since it was never found in non-pregnant women or in men and is absent in the fetal blood. Other investigators reported that oxytocinase exists only in the plasma of pregnant women and monkeys and not in other species (Titus et al., 1960). However, Gilbert and Lake (1964) found that oxytocinase was present in the plasma of laying hens, non-laying hens and cocks in approximately equal amounts. They also reported that the activity of this enzyme decreases at about the time of oviposition and that this decrease coincides with the decrease of oxytocic substances in the pituitary reported by Tanaka and Nakajo (1962). This period of oxytocin release and oxytocinase decrease is shorter than the period of reduced fertility observed by Johnston (1967) and by Hughes (1970). On the basis of the above reports it would appear that the reduction in fertility cannot be attributed entirely to oxytocin.

Other investigators have confirmed the presence of oxytocinase in the plasma of chickens (Brzezinska and Ewy, 1965, 1970); but the period of oxytocinase decrease reported by these authors was longer than that previously reported (Brzezinska, Rzasa and Ewy, 1967). Their observed period of decreased oxytocinase level was about as

long as the period of decreased fertility observed by Johnston (1967) and by Hughes (1970). Figures 1 and 2 show this similarity between plasma levels of oxytocinase and the changes in fertility. If oxytocinase level is inversely proportional to the rate of oxytocin secretion the low fertility observed would occur when the plasma level of oxytocin was high.

Effects of Epinephrine on the Uterus and Fertilization

In the cow epinephrine (adrenalin) injections cause a period of uterine quiescence usually preceded by one large contraction and will also partially or completely inhibit the effect of oxytocin injections (VanDemark and Hays, 1951; Hays and VanDemark, 1953a). An intravenous injection of 2 ml of a 1/1,000 solution of epinephrine within five minutes before natural mating resulted in a nonsignificant increase in conception rate in one group of cows--48% vs. 58% (Hays, VanDemark and Ormiston, 1958). In view of its effect on the uterus one might expect that epinephrine would reduce spermatozoa transport and thereby reduce conception rate instead of increasing it.

Epinephrine has been shown to have an effect on the chicken uterus or shell gland similar to that observed in the cow causing an initial contraction followed by a period of relaxation lasting about 30 minutes (Morash and Gibbs, 1929; McKenney, Essex and Mann, 1932);

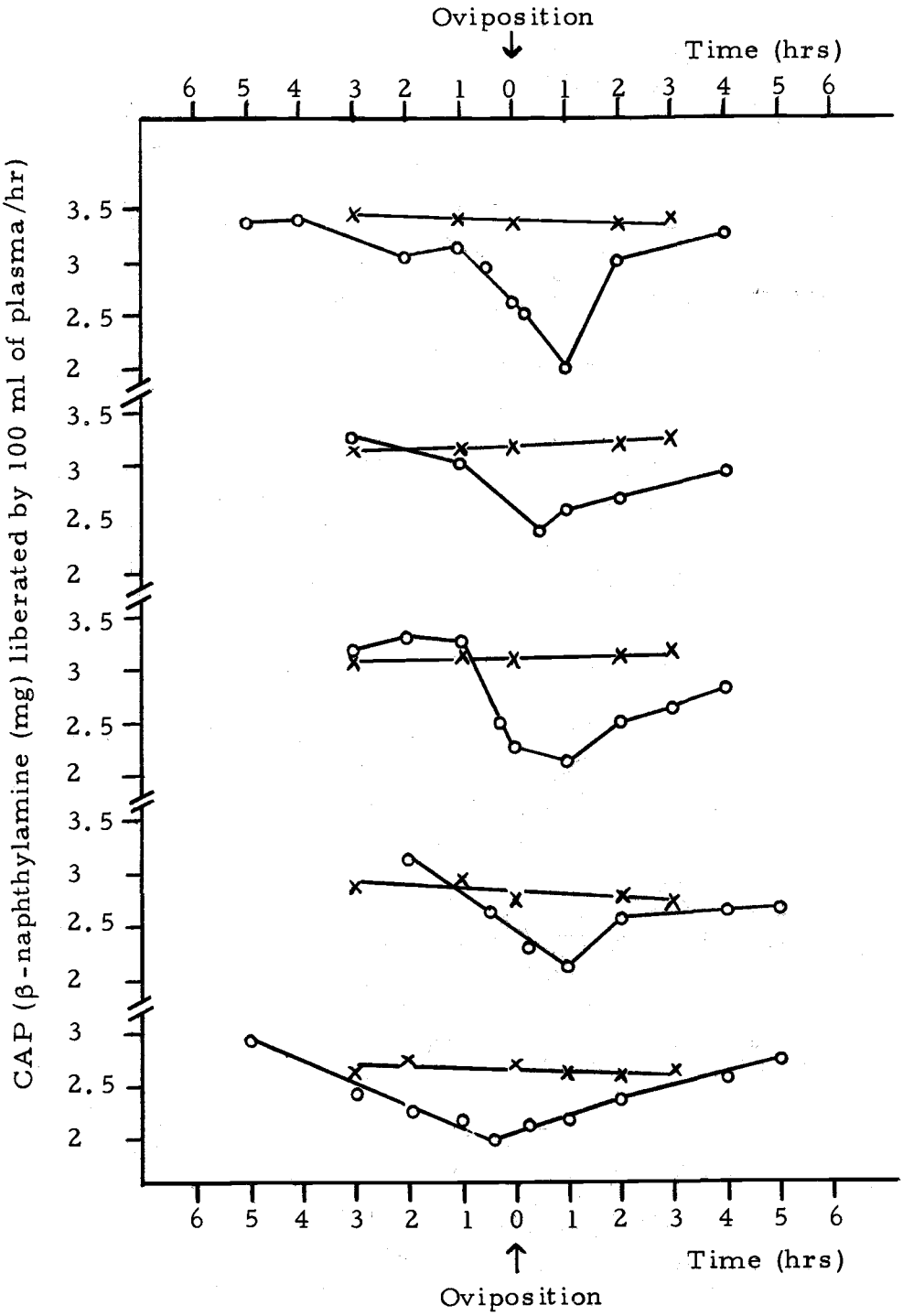

Figure 1. Changes in plasma aminopeptidase activity in hens during oviposition (o-o-o) and interlaying period (x-x-x). Each curve corresponds to an individual hen (Brzezinska et al., 1967).

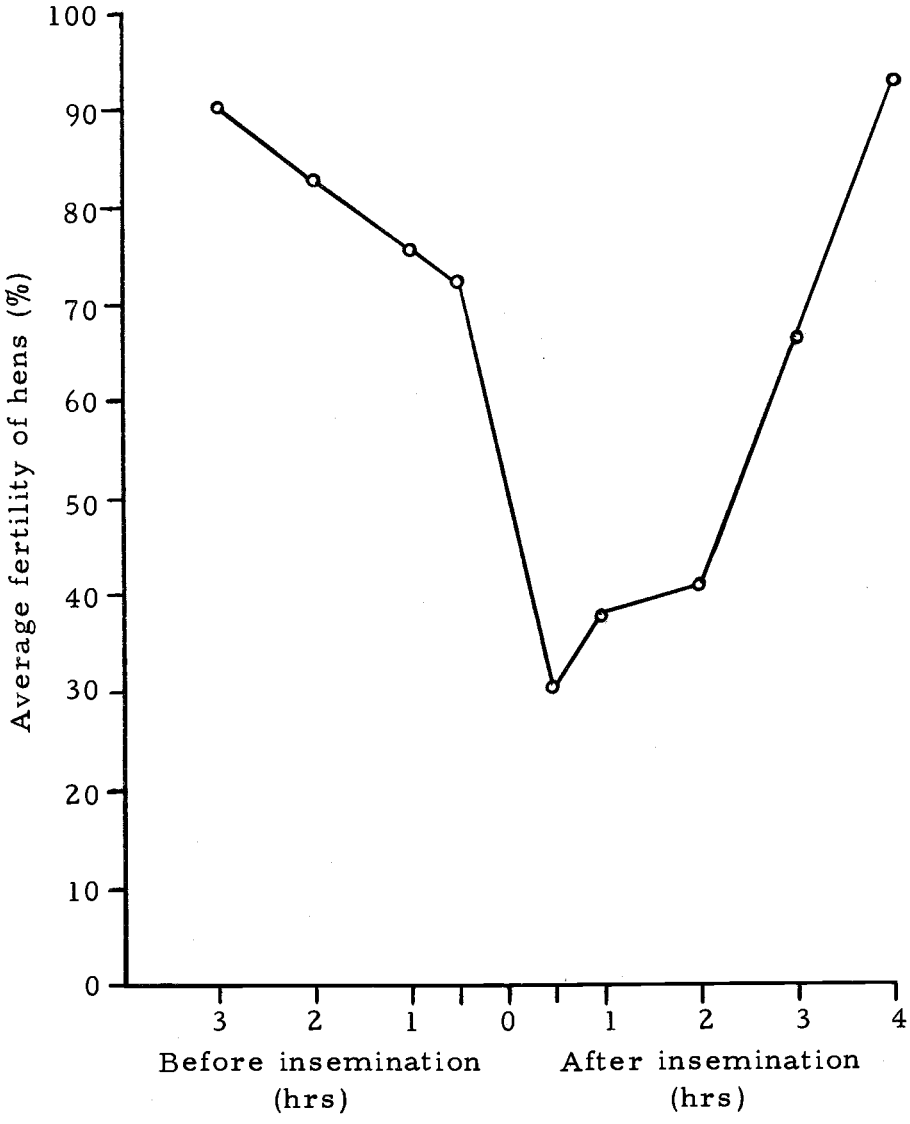

Figure 2. Fertility of hens laying within four hours of insemination (Hughes, 1970). Percent fertility based on eggs laid during a two to nine day period following artificial insemination.

however, one investigator has reported that intramuscular injections of epinephrine prior to insemination had no effect on fertility in chickens (Hawes, 1962).

Effect of Oxytocin on Semen Volume and Concentration of Spermatozoa

Intravenous injections of oxytocin in male rabbits increased the volume of the first ejaculate obtained immediately after injection. The total number of sperm in the first ejaculate following oxytocin injection did not differ significantly from that obtained from saline injected males suggesting that the increase in semen volume was due to increased amounts of seminal fluid. The volume of subsequent ejaculates and total number of spermatozoa obtained over a 30 minute period was not affected by the oxytocin injection. Oxytocin injections significantly reduced the time required to obtain the first ejaculate and significantly increased the total number of ejaculates obtained over a 30 minute period (Kihlstrom and Melin, 1963; Melin and Kihlstrom, 1963).

In bulls, intravenous injection of 3 to 18 units of oxytocin increased ejaculate volume, concentration of spermatozoa and total number of spermatozoa when two ejaculates were collected within five minutes after injection (Milovanov, Bereznev and Gorohov, 1962). It also has been shown that oxytocin is not present in the blood of bulls

which are not sexually excited or in that of bulls which have served five minutes previously. However, oxytocin is present in the blood immediately before and immediately after service with the concentration being greater before than after service, and is directly related to ejaculate volume (Bereznev, 1963). Oxytocin injections increased smooth muscle contractions of testis, epididymis and vas deferens and also increased sperm accumulations in the ampulla of bulls (Bereznev, 1964).

Intravenous injections of oxytocin into rams immediately before collection of semen increased semen volume, concentration and number of spermatozoa per ejaculate within five minutes after injection. However after injections of 35 I. U. of oxytocin per week over a six week period the semen volume and number of spermatozoa per ejaculate declined to the level of non-treated rams. The percentage of abnormal spermatozoa in semen from rams treated with oxytocin increased significantly in the 7th week (Knight and Lindsay, 1970). It has also been shown that oxytocin injections cause an increase in the flow of spermatozoa from the vas deferens of rams (Ewy, Bielanski and Zapletal, 1963).

Reports of investigations concerning the effect of oxytocin on semen quantity and quality in male chickens was not found in the literature.

III. EXPERIMENTAL PROCEDURE

General Procedure

White Leghorn hens individually caged and in their first year of lay were used in all trials concerned with the effects of hormones on females, and were used to determine the fertilizing capacity of males in all but one trial concerned with the effect of oxytocin on males. This exception will be noted in the procedure for individual experiments. The males supplying the semen were maintained in individual cages and housed in the same building as the females. Artificial lighting was provided from 5 a.m. to 7 p.m., giving 14 hours of light per day.

The method of semen collection and artificial insemination was similar to that described by Burrows and Quinn (1939). Mixed semen obtained from at least four cockerels was used in all trials except those concerned with the effect of oxytocin on males. The semen was used as soon as possible after collection with the maximum time between collection and insemination being 45 minutes.

The number of hens per treatment that produced eggs from which data were obtained is stated in the tables summarizing the results of each individual experiment (see Appendix). In the majority of the experiments an equal number of hens were used in each treatment. However, due to the failure of some hens to lay or to mortality

the number of hens per treatment from which data were obtained may vary.

All intravenous injections were made in the brachial vein. Subcutaneous injections of oxytocin were made in the loose skin on the dorsal surface of the cervical region. Subcutaneous injections of epinephrine were made in the pectoral region. The oxytocin used in experiments 1 and 5 was P. O. P. (Oxytocin Injection), 20 U. S. P. units per ml, Armour-Baldwin Laboratories; and that used in experiments 2, 3, and the first trial of experiment 4 was Veterinary Oxytocin Injection, 20 U. S. P. units per ml, Bio-Ceutic Laboratories. In all other instances the oxytocin used was Synthetic Oxytocin, 10 I. U. per ml, Nutritional Biochemicals Corporation. The vasopressin used was Vasopressin (Synthetic Arginine Vasopressin), 1,000 I. U. per ml, Nutritional Biochemicals Corporation. The epinephrine used was Epinephrine Injection, 1:1,000 solution, Haver-Lockhart Laboratories.

Percentages of fertility were usually based on eggs laid from the second through the ninth day following insemination. Exceptions to this will be noted in the procedure for individual experiments. This study was primarily concerned with fertility but data on hatchability also were recorded. Hatchability in this study was defined as the percentage of fertile eggs that hatched. After incubation for 18 days the eggs were candled and the fertile eggs were transferred to

hatching trays. Eggs which appeared infertile when candled were broken and observed macroscopically for evidence of embryonic development. Chicks were removed from the incubator on the 22nd day and the unhatched eggs were broken to recheck their fertility.

Fertility and hatchability data were analyzed by analysis of variance after arcsin transformation of the percentage values. Differences between treatment means were tested either by Student's t-test or by Least Significant Difference (Snedecor and Cochran, 1967).

In several experiments hormone injections were used in an attempt to create a uterine environment that might be comparable to that in the oviduct near the time of laying. This procedure is hereafter referred to as "simulated laying." The hens used in these experiments did not have hard-shelled eggs in their uteri; therefore "simulated laying" does not involve the actual laying of a hard-shelled egg.

Individual Experiments

Experiment 1. Effect of Acetic Acid and Chlorobutanol on Fertilization

Since some of the oxytocin used in these experiments contained 0.25% acetic acid and 0.50% chlorobutanol it was thought desirable to determine the effect of these compounds on fertilization rate when

injected intravenously. One group of hens was given an intravenous injection of 5 I. U. of oxytocin immediately before insemination. A second group was given an intravenous injection of 0.25 ml of a 0.85% saline solution containing 0.25% acetic acid and 0.50% chlorobutanol immediately before insemination. The total volume injected (0.25 ml) was the same in both groups. A third group received no injections. All hens had laid at least four hours before insemination and each received 0.03 ml of undiluted mixed semen.

Experiment 2. Dosage of Oxytocin and Oviposition

To determine the amount of oxytocin needed to give a consistent oviposition response and to compare the effects of different levels of oxytocin on fertility and hatchability three groups of hens were given intravenous injections of either 1, 3 or 5 I. U. of oxytocin immediately before insemination. A fourth group of hens was inseminated but received no injections.

All hens had laid at least two hours prior to treatment and each was inseminated with 0.05 ml of mixed semen. Eggs were saved and incubated in groups of 2 to 9 and 10 to 17 days following insemination.

Experiment 3. Oxytocin Injections Before Insemination

This experiment was an attempt to create a uterine environment

similar to that of laying at various times before insemination in the absence of a hard-shelled egg.

Hens that had laid at least two hours prior to treatment were divided into six groups. One group was inseminated and received no further treatment. The remaining five groups were given intravenous injections of 5 I. U. of oxytocin and then inseminated immediately, 30 minutes, one hour, two hours and five hours after the oxytocin administration.

Each hen was inseminated with 0.05 ml of mixed semen. Eggs were saved and incubated in groups of 2 to 9 and 10 to 17 days following insemination.

Experiment 4. Injections After Insemination

The following five trials were attempts to create a uterine environment similar to that of laying at various times after insemination in the absence of a hard-shelled egg. The purpose of this was to try to separate the effects on fertility caused by laying after insemination into those caused by the hard-shelled egg itself and those possibly caused by the hormones that induce oviposition. To avoid the effect of a hard-shelled egg in the uterus only hens that already had laid on the day of treatment were used. Furthermore, to avoid the adverse effect on fertility observed when hens lay within two hours prior to insemination (Hughes, 1970) only hens that had laid at least

two hours prior to treatment were used. Two hormones of the posterior pituitary gland, oxytocin and arginine vasopressin, were used either singly or in combination.

1. Oxytocin. A trial was conducted in duplicate using six groups of hens that had laid at least two hours prior to treatment. Each hen was inseminated with 0.05 ml of mixed semen. One group was inseminated and received no further treatment. The remaining five groups received an intravenous injection of 5 I. U. of oxytocin immediately, 30 minutes, one hour, two hours and five hours after insemination respectively.

2. Vasopressin. Hens that had laid at least two hours previously were randomly assigned to one of four groups. One group was inseminated and received no further treatment. The remaining three groups received an intravenous injection of 3 I. U. of vasopressin immediately, 30 minutes and one hour after insemination respectively. Each hen was inseminated with 0.05 ml of mixed semen.

3. Oxytocin-Vasopressin Mixture. A mixture of oxytocin and vasopressin was used to test the possibility that a combination of the two hormones might cause a greater reduction in fertility than either one alone. The design of this trial was similar to the one just described except for the hormones used and an additional control group was added. The treated hens received an intravenous injection of a saline solution containing 2.5 I. U. of oxytocin and 1.5 I. U. of

vasopressin. The additional control group received an intravenous injection of 0.3 ml of a 0.85% saline solution immediately after insemination.

4. Vasopressin and Oxytocin-Vasopressin Mixture. This trial was conducted to gain additional data on the effects of vasopressin and to separate its effects from those caused by the oxytocin when the two hormones were given in combination. The control group of hens was given an intravenous injection of 0.3 ml of a 0.85% saline solution immediately after insemination. The next three groups received an intravenous injection of 3 I.U. of vasopressin immediately, 30 minutes and one hour after insemination respectively. The last three groups received an intravenous injection immediately after insemination of either a saline solution containing 2.5 I.U. of oxytocin and 1.5 I.U. of vasopressin, 2.5 I.U. or 5.0 I.U. of oxytocin. All hens had laid at least three hours before treatment and each hen was inseminated with 0.05 ml of mixed semen.

5. Multiple Injections of Oxytocin. As pointed out in the Review of Literature there is a gradual decrease in the plasma levels of oxytocinase beginning one to two hours before oviposition (Brzezinska, Rzasa and Ewy, 1967). If oxytocinase level is inversely proportional to oxytocin level, there is a gradual increase in the plasma concentration of oxytocin beginning one to two hours prior to oviposition and reaching a maximum at the time of oviposition. Studies on plasma

levels of oxytocin indicate that oviposition is initiated by a sudden increase of oxytocin in the plasma. This trial was an attempt to cause a slow release of oxytocin followed by a sudden surge of this hormone. The control group of hens received an intravenous injection of 0.3 ml of a 0.85% saline solution immediately after insemination. Two groups received 3 I.U. of oxytocin either intravenously or subcutaneously immediately after insemination. A fourth group received subcutaneous injections of 1 I.U. of oxytocin immediately and 30 minutes after insemination followed by an intravenous injection of 3 I.U. of oxytocin one hour after insemination. The last group received subcutaneous injections of 1 I.U. of oxytocin immediately, 30 minutes and one hour after insemination followed by an intravenous injection of 3 I.U. of oxytocin two hours after insemination. All hens had laid at least three hours prior to treatment and each hen was inseminated with 0.05 ml of mixed semen.

Experiment 5. Direct Effect of Hormones on Semen

This trial was conducted to determine if oxytocin or epinephrine when added directly to semen had an effect on spermatozoa which lowered their capacity to fertilize eggs. Since the oxytocin used contained 0.25% acetic acid and 0.50% chlorobutanol the effects of adding these compounds to semen were also studied. The four semen preparations used were as follows:

1. One ml of semen diluted with 0.1 ml (2 I.U.) of oxytocin.
2. One ml of semen diluted with 0.1 ml of a 0.85% saline solution containing 0.25% acetic acid and 0.50% chlorobutanol.
3. One ml of semen diluted with 0.125 ml of a 1:1,000 solution of epinephrine (0.125 mg epinephrine per ml of semen).
4. Undiluted semen.

The four semen preparations were made from a common pool of mixed semen. All hens had laid at least four hours prior to insemination and each hen received 0.03 ml from one of the four semen preparations.

Experiment 6. Counteracting the Effect of Oxytocin with Epinephrine

This trial was conducted to determine if epinephrine injections would counteract the adverse effect of oxytocin injections on fertilization rate. The four treatments were as follows:

1. Intravenous injection of 5 I.U. of oxytocin immediately after insemination.
2. Subcutaneous injection of 1 mg of epinephrine 20 minutes before insemination.
3. Subcutaneous injection of 1 mg of epinephrine 20 minutes before insemination and an intravenous injection of 5 I.U. of oxytocin immediately after insemination.

4. Intravenous injection of 0.5 ml of a 0.85% saline solution immediately after insemination.

All hens had laid at least three hours prior to treatment and each hen received 0.03 ml of mixed semen.

Experiment 7. Counteracting the Effect of Hard-shelled Eggs with Epinephrine

Since epinephrine injections cause relaxation of the uterus it was thought that this hormone might counteract the adverse effect on fertilization caused by the presence of a hard-shelled egg in the uterus at the time of insemination. To test this possibility one group of hens was given an intravenous injection of 0.5 mg of epinephrine (0.5 ml of a 1:1,000 solution) 2 to 20 minutes before insemination. A second group was inseminated with semen containing 0.125 mg of epinephrine per ml and a third group was inseminated with undiluted semen and received no further treatment. All hens had hard-shelled eggs in their uteri at the time of insemination and each hen received 0.03 ml of semen. The semen used in each group was obtained from a common pool of mixed semen.

Experiment 8. The Effect of Oxytocin Injections of Males on Volume and Fertilizing Capacity of Semen

The data on semen characteristics were analyzed by analysis of variance and differences between treatment means were tested by

Least Significant Difference (Snedecor and Cochran, 1967). Fertility and hatchability data were analyzed by the methods previously described.

White Leghorn and New Hampshire cockerels were ejaculated two days prior to treatment and data were obtained on semen volume and spermatozoa concentration. The males were then divided into four groups according to semen volume so that each group produced about the same total volume of semen and contained approximately the same number of high- and low-volume males. The four groups of ten males each received the following treatments:

1. Uninjected controls.
2. Intravenous injection of 0.5 ml of a 0.85% saline solution.
3. Intravenous injection of 1 I.U. of oxytocin.
4. Intravenous injection of 5 I.U. of oxytocin.

The time interval between injections and semen collections varied from 10 to 15 minutes. After measuring semen volume three White Leghorn hens were inseminated with 0.05 ml of semen from each male. Fertility and hatchability were determined in the manner previously described. Concentration of spermatozoa was determined by hemacytometer counts. Packed sperm volume was also measured as an estimate of the concentration of spermatozoa (Arscott and Kuhns, 1969).

Two trials were conducted using cockerels which had not been trained for artificial ejaculation. In the first trial 12 White Leghorn cockerels were randomly divided into two groups. One group received no treatment prior to ejaculation and the other group received an intravenous injection of 2.5 I.U. of oxytocin two to five minutes prior to ejaculation. After measuring semen volume two White Leghorn hens were inseminated with 0.05 ml of semen from each male. Fertility and hatchability were determined from eggs laid on the second through the tenth day following insemination. In this and the following trial concentration of spermatozoa was estimated by packed sperm volume only.

A second trial was conducted in the same manner using six Cornish cockerels (three per group). Three days later these same Cornish cockerels were assigned to the treatment opposite from that which they had originally received. Three Delaware hens were inseminated with 0.05 ml of semen from each male and fertility and hatchability were based on eggs laid on the second through the ninth day following insemination.

Two trials were conducted using wider ranges of oxytocin levels. White Leghorn cockerels were ejaculated two days prior to treatment and semen volume was measured for each male. The males were divided into four groups according to semen volume, as described in the first experiment. In the first trial the four groups of

five males received the following treatments:

1. Intravenous injection of 0.5 ml of a 0.85% saline solution.
2. Intravenous injection of 0.25 I. U. of oxytocin.
3. Intravenous injection of 1.0 I. U. of oxytocin.
4. Intravenous injection of 5.0 I. U. of oxytocin.

The volume of each injection was 0.5 ml and the time interval between injection and ejaculation varied from two to seven minutes. After measuring semen volume three White Leghorn hens were inseminated with 0.05 ml of semen from each male. Fertility and hatchability were based on eggs laid on the second through the ninth day following insemination. Concentration of spermatozoa was estimated by packed sperm volume only.

In the second trial the four groups of six males each received the following treatments:

1. Intravenous injection of 0.25 ml of a 0.85% saline solution.
2. Intravenous injection of 0.05 I. U. of oxytocin.
3. Intravenous injection of 0.25 I. U. of oxytocin.
4. Intravenous injection of 1.00 I. U. of oxytocin.

The volume of each injection was 0.25 ml and the time interval between injection and ejaculation varied from one to six minutes. After measuring semen volume three White Leghorn hens were inseminated with 0.05 ml of semen from each male. Fertility and hatchability were based on eggs laid on the second through the ninth day following

insemination. Concentration of spermatozoa as determined both by hemacytometer counts and packed sperm volume also was recorded.

IV. RESULTS

Effect of Acetic Acid and Chlorobutanol Injections on Fertility and Hatchability

Some of the oxytocin solutions used in this study contained 0.25% acetic acid and 0.50% chlorobutanol. Therefore it was thought desirable to determine if injections of these compounds would influence fertility or hatchability and thereby influence the results of experiments involving injections of oxytocin.

The results of this experiment are presented in Table 2 and show that intravenous injections of a saline solution containing 0.25% acetic acid and 0.50% chlorobutanol immediately before insemination had no effect on either fertility or hatchability.[2] The amount of acetic acid and chlorobutanol injected was equal to the amount of these compounds present in 5 I. U. of oxytocin. Therefore, the significant reduction in fertility ($P < .01$) observed when 5 I. U. of oxytocin was injected immediately before insemination was due to the oxytocin alone. In subsequent experiments it was assumed that the presence of acetic acid and chlorobutanol in the oxytocin injections had no influence on the fertility and hatchability results.

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[2] Tables 1 through 22 are presented in the Appendix.

Dosage of Oxytocin as Related to Fertility

The results of intravenous injections of 1, 3 and 5 I. U. of oxytocin immediately before insemination on fertility and hatchability are presented in Table 3. Although 1 I. U. of oxytocin tended to cause a reduction in fertility of eggs laid two to nine days following insemination the reduction was not statistically significant. Both 3 and 5 I. U. of oxytocin caused a significant ($P < .01$) reduction in fertility of eggs laid two to nine days following insemination and the degree of reduction was comparable for both treatments. Fertility of eggs laid 10 to 17 days following insemination was low and not significantly affected by any of the levels of oxytocin used. Hatchability was not significantly affected by any of the treatments.

Injection of 5 I. U. of oxytocin caused a significant reduction in the average duration of fertility (Table 4). If the data on infertile hens were excluded from the calculations the average duration of fertility was not affected by any of the levels of oxytocin used. This shows that the reduction in average duration of fertility caused by 5 I. U. of oxytocin was due mainly to infertile hens while those hens that were fertile exhibited a normal duration of fertility. From this one might expect that the observed reduction in fertility was primarily a result of infertile hens. Examination of the data showed this to be true in the group receiving 5 I. U. of oxytocin but not in the group receiving 3 I. U. of oxytocin.

Although 3 I. U. of oxytocin caused a reduction in fertility similar to that caused by 5 I. U. of oxytocin, the latter was more consistent in causing premature oviposition. In several subsequent experiments oxytocin was used in an attempt to create a uterine environment that might be comparable to that in the oviduct near the time of laying. As pointed out in the Experimental Procedure this is referred to as "simulated laying." Since the level of 5 I. U. was more consistent in producing premature oviposition this level was used in the majority of these experiments.

Effect of Oxytocin Injections Before Insemination on Fertility and Hatchability

Intravenous injections of 5 I. U. of oxytocin immediately before insemination caused a significant reduction in the fertility ($P < .01$) of eggs laid 2 to 9 and 10 to 17 days following insemination (Table 5). The same treatment given 30 minutes, one hour, two hours or five hours before insemination had no significant effect on fertility. The purpose of this experiment was to "simulate laying" at various times before insemination and to compare the resulting fertility with that observed when natural lay occurs before insemination. Figure 3 shows that the fertility patterns with "simulated laying" and natural laying at various times before insemination were similar but the reduction in fertility was not of the same magnitude. Hatchability was not significantly affected by any of the treatments.

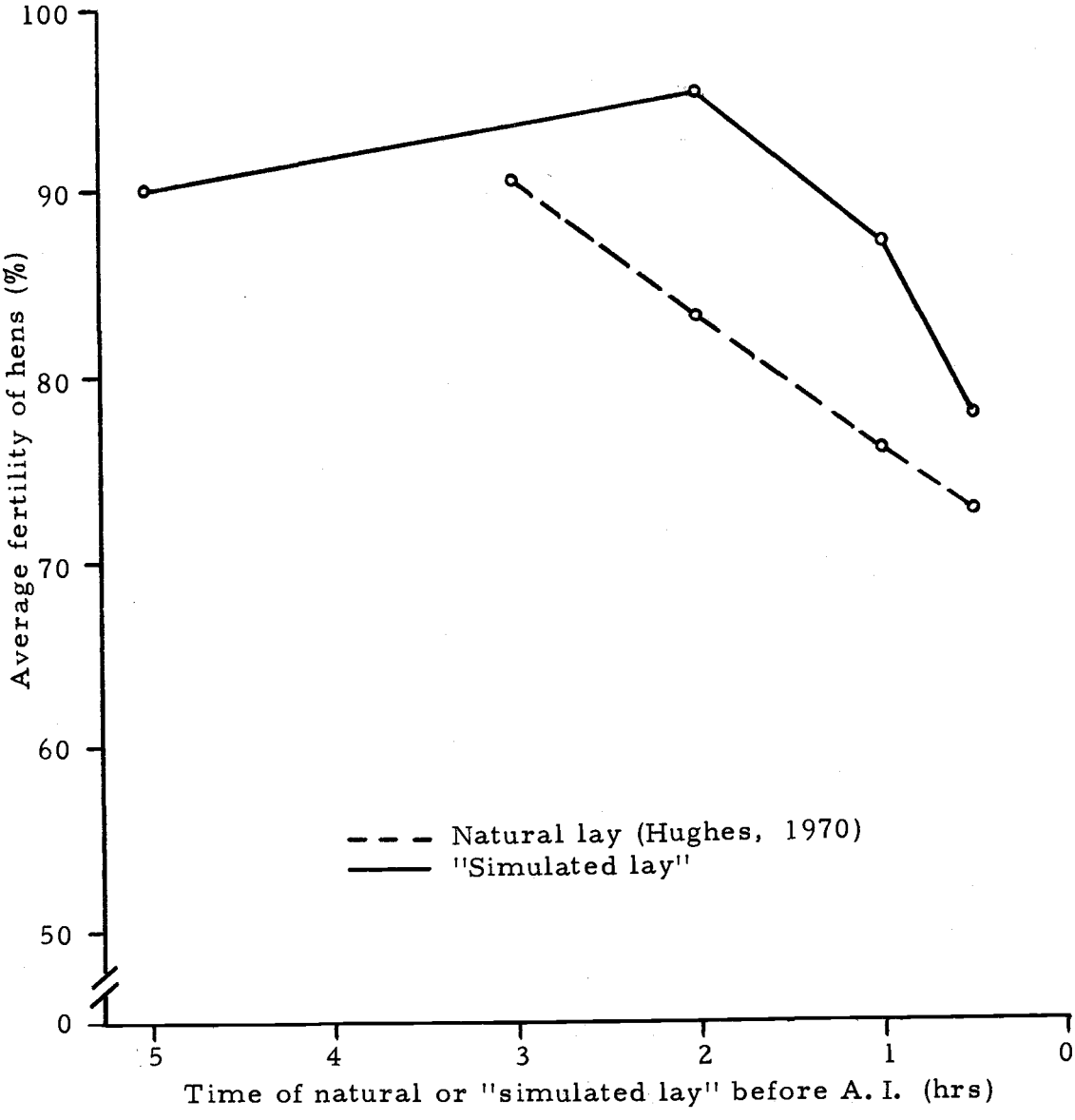

Figure 3. Fertility of hens for a two to nine day period following artificial insemination after natural oviposition or "simulated lay" resulting from injecting oxytocin in hens with no egg in the oviduct.

Average duration of fertility was significantly reduced ($P < .01$) when the oxytocin injection was given immediately before insemination but was not affected when the injections were given at other times (Table 6). With the data on infertile hens excluded from the calculations average duration of fertility was still significantly reduced in the group receiving oxytocin injections immediately before insemination. This is not in agreement with the results of the previous experiment in which the reduction in duration of fertility was due primarily to infertile hens.

Effect of Hormone Injections After Insemination on Fertility and Hatchability

Oxytocin

The fertility and hatchability results of the first trial of this experiment in which intravenous injections of 5 I. U. of oxytocin were used to "simulate laying" at various times after insemination are presented in Table 7. Oxytocin injections immediately or 30 minutes after insemination caused a significant reduction in fertility ($P < .01$). Small and statistically nonsignificant reductions in fertility occurred when oxytocin was injected either one or two hours after insemination; but when the oxytocin injection was made five hours after insemination there was no reduction in fertility. Figure 4 shows that there was a similarity between the fertility pattern with "simulated

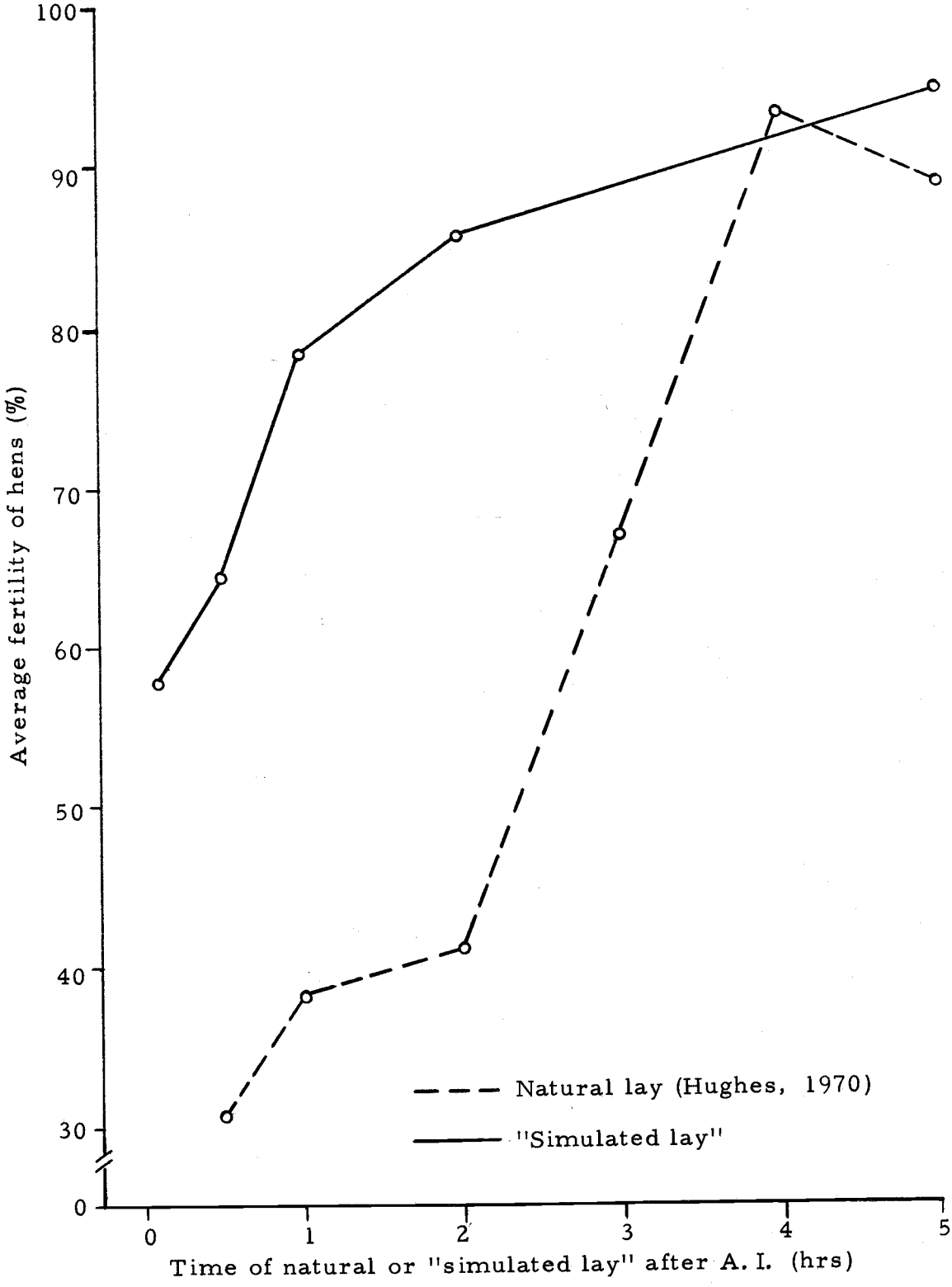

Figure 4. Fertility of hens for a two to nine day period following artificial insemination before natural oviposition or "simulated lay" resulting from injecting oxytocin in hens with no egg in the oviduct.

laying" and natural laying at various times after insemination; however the reduction in fertility caused by natural laying was much greater than that caused by the "simulated laying" by means of oxytocin injections. With natural laying after insemination there were at least two factors which may have affected fertilization, the presence of the hard-shelled egg in the uterus and the hormones that initiated oviposition. With "simulated laying" the effect of the hard-shelled egg was not present since the hens used did not have hard-shelled eggs in their uteri at the time of insemination. Considering these facts the above results suggest that there was an additive adverse effect on fertility with natural laying caused by the presence of the hard-shelled egg and the hormones that initiate oviposition. Evidence has been presented indicating that the observed reduction in fertility is not entirely due to the presence of an egg in the uterus at the time of insemination. Hatchability was not significantly affected by the oxytocin injections in this trial.

Vasopressin

Since the oxytocin injections did not reduce fertility to the level caused by natural laying, arginine vasopressin was used to "simulate laying" after insemination.

Intravenous injections of 3 I. U. of arginine vasopressin immediately, 30 minutes or one hour after insemination tended to

reduce fertility but the degree of reduction was not statistically significant (Table 8). Arginine vasopressin did not reduce fertility as much as oxytocin (see Table 7). The shorter the time interval between insemination and injection the greater was the reduction in fertility; this is in agreement with the results from oxytocin injections and natural lay. Hatchability was not affected by the injections of arginine vasopressin.

Oxytocin-Vasopressin Mixture

In this trial a mixture containing 2.5 I. U. of oxytocin and 1.5 I. U. of arginine vasopressin was used to test the possibility that a combination of the two hormones may cause a greater reduction in fertility than either one alone when used to "simulate laying."

Table 9 shows that intravenous injections of the oxytocin-vasopressin mixture caused a significant reduction in fertility when given immediately after insemination ($P < .01$) or 30 minutes after insemination ($P < .05$) as compared to the uninjected controls. However in comparison to the saline injected controls fertility was significantly reduced only when the oxytocin-vasopressin mixture was injected immediately after insemination. The combination of the two hormones caused a greater reduction in fertility than vasopressin alone when injected immediately or 30 minutes after insemination (see Tables 8 and 9). In comparison to the results from oxytocin

injections (see Tables 7 and 9) the oxytocin-vasopressin mixture caused a slightly greater reduction in fertility when injected immediately after insemination but not when injected at other times. Injection of saline solution immediately after insemination had no significant effect on fertility. Hatchability was not affected by any of the treatments.

Vasopressin and Oxytocin-Vasopressin Mixture

This trial was a further study of the effects of intravenous injections of vasopressin and oxytocin-vasopressin mixture after insemination on fertility; and an attempt to separate the effects of the mixture into those caused by oxytocin and those caused by vasopressin.

The fertility and hatchability results are presented in Table 10. The fertility results of vasopressin injections alone are similar to those obtained previously (see Table 8). Although vasopressin injections immediately after insemination caused a significant reduction in fertility ($P < .05$) the reduction was not as large as that obtained with either 2.5 or 5 I.U. of oxytocin.

Intravenous injections of the oxytocin-vasopressin mixture immediately after insemination caused a significant reduction in fertility ($P < .01$). This reduction in fertility was greater than that observed in the previous trial and greater than that caused by 2.5 or

5 I.U. of oxytocin alone. However the difference between the fertility reduction caused by the oxytocin-vasopressin mixture and that caused by either 2.5 or 5 I.U. of oxytocin was not statistically significant. Although the data suggest a combination of oxytocin and vasopressin caused a greater reduction in fertility than either hormone alone this cannot definitely be concluded. Hatchability was not significantly affected by any of the treatments.

Multiple Injections of Oxytocin

Attempts to simulate a slow release of oxytocin before oviposition and a sudden surge of oxytocin to initiate oviposition at one and two hours after insemination are shown in Table 11 (treatments 4 and 5 respectively). Both treatments caused small and nonsignificant reductions in fertility and these reductions were similar to those observed when single injections of oxytocin were given at one and two hours after insemination (see Table 7). Intravenous and subcutaneous injections of oxytocin immediately after insemination caused significant reductions in fertility ($P < .01$ and $P < .05$) respectively. The intravenous injections tended to cause a greater reduction in fertility than did the subcutaneous injections but the difference between the two methods of injection was not statistically significant. Hatchability was not significantly affected by any of the treatments.

Direct Effect of Hormones on Fertilizing Capacity of Semen

Hens inseminated with semen containing 2 I. U. of oxytocin per ml of semen were 78.6% fertile and those with 0.125 mg of epinephrine per ml were 85.0% fertile as compared to 96.8% for hens inseminated with undiluted semen (Table 12). However, neither oxytocin nor epinephrine in the semen caused a statistically significant reduction in fertility. That addition of oxytocin to semen causes a reduction in fertility has previously been reported by Hughes and Parker (1970) who observed a significant ($P < .01$) reduction in fertility when hens were inseminated with semen containing 2 I. U. of oxytocin per ml.

The oxytocin used in this experiment contained 0.25% acetic acid and 0.50% chlorobutanol. The same levels of these compounds in a saline solution had no effect on fertility when added to semen.

Hatchability was somewhat lower in the hens inseminated with semen containing epinephrine or acetic acid and chlorobutanol than in the hens inseminated with semen containing oxytocin or undiluted semen. No explanation can be given for these differences, and furthermore the differences were not statistically significant.

Counteracting the Effect of Oxytocin with Epinephrine

Intravenous injections of hens without hard-shelled eggs in their

uteri with 5 I. U. of oxytocin immediately after insemination already have been shown to cause a significant reduction in fertility (Table 10). Similar fertility results were obtained when this same treatment was preceded by a subcutaneous injection of 1 mg of epinephrine 20 minutes before insemination (Table 13). The epinephrine injections alone had a tendency to improve fertility but were not effective in counteracting the adverse effect of oxytocin injections. The high fertility rate (100%) of hens injected with epinephrine 20 minutes before insemination was not the result of a short egg production period, as over half of the hens in this group continued to lay throughout the nine day period during which eggs were saved for incubation. Although the difference in fertility rates between the epinephrine injected group and the saline injected controls was not statistically significant, the fact that the epinephrine injected hens were 100% fertile suggests a need for further investigation in this area. Hatchability was not affected by any of the treatments.

Egg production was significantly reduced ($P < .05$) in the two groups of hens receiving epinephrine injections. This is in agreement with the results of Sykes (1955b) who also reported that subcutaneous injections of epinephrine lowered subsequent egg production.

Counteracting the Effect of Hard-shelled Eggs with Epinephrine

Insemination of hens when a hard-shelled egg was present in

their uteri resulted in a relatively low rate of fertilization (Table 14). Intravenous injections of 0.5 mg of epinephrine 2 to 20 minutes before insemination or addition of 0.125 mg of epinephrine per ml of semen failed to overcome the adverse effect of the hard-shelled egg. With both of these treatments epinephrine had a tendency to cause a greater reduction in fertility than that caused by the presence of the hard-shelled egg alone. This is not in agreement with the previous results showing that subcutaneous injections of epinephrine 20 minutes before insemination tended to increase fertility in hens that did not have hard-shelled eggs in their uteri. There was no significant difference in hatchability among the treatments.

Contrary to our previous results with subcutaneous injections of epinephrine, the intravenous injections of epinephrine had no effect on subsequent egg production (Tables 13 and 14). The difference was probably due to the method of injection. Sykes (1955b) observed a 20% drop in egg production following subcutaneous injections of epinephrine while Hawes (1962) reported that intramuscular injections of epinephrine had no adverse effect on subsequent egg production.

The Effect of Oxytocin Injections of Males on Volume and Fertilizing Capacity of Semen

In the first trial intravenous injections of 5 I.U. of oxytocin in males 10 to 15 minutes before artificial ejaculation caused a significant ($P < .01$) reduction in the average semen volume obtained (Table 15).

Intravenous injections of 1 I. U. of oxytocin also had a tendency to decrease semen volume but the decrease was not statistically significant when the data were analyzed using the actual volumes obtained. If semen volume obtained after treatment was analyzed on the basis of that obtained two days prior to treatment, 1 I. U. of oxytocin did cause a significant decrease ($P < .05$) in semen volume. Concentration of sperm, as determined by hemacytometer counts, was not affected by intravenous injections of either 1 or 5 I. U. of oxytocin. When packed sperm volume was used as a measure of the concentration of sperm the results indicated that injections of 5 I. U. of oxytocin caused a significant ($P < .05$) increase in the concentration of sperm; however upon examination of the data it was found that one semen sample had an exceptionally high packed sperm volume (19.5%) and the significance obtained may have been due to this one semen sample. Neither 1 I. U. nor 5 I. U. of oxytocin had a significant effect on the total number of sperm obtained per ejaculate. From the data in Table 15 one would suspect that the oxytocin injections did cause a significant decrease in the total number of sperm per ejaculate; however as might be expected from the lack of significance, the high value recorded for the control group was due to one male that produced a large volume of highly concentrated semen. When the data on this male were omitted from the calculations, the average value of total number of sperm per ejaculate for the control group was 1.69 billion. Since 5 I. U. of oxytocin caused

a reduction in semen volume but not in total number of sperm per ejaculate the reduction in semen volume was probably due to a decrease in the amount of seminal plasma in the semen. Injections of saline had no significant effect on semen volume, concentration of sperm or total number of sperm per ejaculate. Therefore any differences obtained in this and subsequent trials with oxytocin injections were assumed to be due to the oxytocin and not to the injections per se. There was no significant effect on fertilizing capacity of the semen or hatchability of fertilized eggs caused by injections of saline or oxytocin (Table 16).

Injections of 2.5 I.U. of oxytocin two to five minutes prior to artificial ejaculation in Leghorn males that had not been trained for artificial ejaculation caused a significant decrease ($P < .05$) in semen volume and a significant increase ($P < .05$) in the concentration of sperm as measured by packed sperm volume (Table 17). The same treatment with Cornish males that had not been trained for artificial ejaculation had no significant effect on either semen volume or concentration of sperm; however concentration of sperm did show a tendency to increase. The previous results showed that oxytocin injections did not increase the total number of sperm per ejaculate; therefore the apparent increase in the concentration of sperm in semen from the Leghorn males injected with oxytocin was probably due to a decrease in the amount of seminal plasma in the semen. Injections of

2.5 I. U. of oxytocin in Leghorn and Cornish males did not have a significant effect on fertilizing capacity of the semen or hatchability of fertilized eggs (Table 18).

The above results show that oxytocin injections in male chickens caused a decrease in semen volume and had no effect on total number of sperm per ejaculate. This is not in agreement with the results of oxytocin injections in rabbits, bulls and rams. As was pointed out in the Review of Literature oxytocin injections increased semen volume in rabbits and increased semen volume, concentration of sperm and number of spermatozoa per ejaculate in rams and bulls. On the basis of body weight the amounts of oxytocin used in the above trials with cockerels were considerably higher than those used with rams and bulls. Therefore it was thought that lower doses of oxytocin might have a different effect on semen production in cockerels than that obtained in the previous trials. Two additional trials were conducted to test this possibility.

In the first trial oxytocin injections of 0.25, 1.0 and 5.0 I. U. were used. The time interval between injection and ejaculation varied from two to seven minutes. All three levels of oxytocin had a tendency to lower semen volume and to increase the concentration of sperm (Table 19). However the effects on semen volume and concentration of sperm were not statistically significant. Fertilizing capacity of the semen was not affected by the oxytocin injections (Table 20). The

statistical analysis of the hatchability data indicated that injections of 0.25 I. U. of oxytocin caused a significant ($P < .05$) reduction in hatchability as compared to injections of 5 I. U. of oxytocin (Table 20); however, there were no significant differences in hatchability between the control group and the oxytocin injected groups.

In the second trial oxytocin injections of 0.05, 0.25 and 1.0 I. U. were used. The time interval between injection and ejaculation varied from one to six minutes. The results on semen production are presented in Table 21. Injections of 0.05 I. U. of oxytocin had no effect on semen volume, concentration of sperm or total sperm per ejaculate. Semen volume was slightly reduced by injections of 0.25 I. U. of oxytocin and significantly reduced by injections of 1.0 I. U. of oxytocin. Injections of 0.25 and 1.0 I. U. of oxytocin had a tendency to increase the concentration of sperm (not statistically significant) but neither level had an apparent effect on total number of sperm per ejaculate. Neither fertilizing capacity of the semen nor hatchability of fertilized eggs was significantly affected by the oxytocin injections (Table 22).

V. DISCUSSION

For a considerable period of time, dating from the time of the experiments of Moore and Byerly (1942), Gracewski and Scott (1943) and Parker (1945), it has been known that the presence of a hard-shelled egg in the uterus of the hen at the time of insemination had an adverse effect on fertilization. Results of later research showed that the reduced fertility of hens artificially inseminated prior to laying was not due entirely to the presence of a hard-shelled egg in the uterus at the time of insemination (Johnston and Parker, 1970; Hughes, 1970). The possibility that the posterior pituitary hormones that induce oviposition contributes to this impairment of fertility was demonstrated by Hughes and Parker (1970).

In some of the experiments reported herein hens without hard-shelled eggs in their uteri were injected with posterior pituitary hormones at various times before or after insemination to observe the effects of these hormones on fertility in the absence of the hard-shelled-egg effect. The fact that posterior pituitary hormone injections caused a pattern of reduced fertility similar to that observed when hens lay before or after insemination (Johnston and Parker, 1970; Hughes, 1970) suggests that these hormones are responsible, at least partially, for the fertility reduction associated with laying near the time of insemination.

Since the hormone injections after insemination did not reduce fertility as much as laying after insemination it is tempting to conclude that the presence of the hard-shelled egg *per se* contributes to the reduction in fertility observed when hens lay shortly after insemination. On the other hand, hormone injections before insemination also did not reduce fertility as much as laying before insemination. Since the hens laying before insemination did not have hard-shelled eggs in their uteri at the time of insemination, the fertility difference between those injected with hormones before insemination and those laying before insemination obviously cannot be attributed to the presence of a hard-shelled egg. This indicates that the hormone injections did not create a uterine environment exactly like that associated with laying an egg, as was anticipated. Only a limited number of hormones, dosage levels and injection times were employed in these experiments. Perhaps with different combinations of hormones, dosage levels and injection times it might be possible to produce reductions in fertility more nearly like those observed when hens lay near the time of insemination.

Since fertility was reduced more when laying or hormone injections occurred after insemination than when they occurred before insemination it seems that the fertility-reducing-effect is short lived and may depend on the length of time that the sperm are subjected to the changes taking place in the uterus near the time of laying. The

fact that addition of oxytocin to semen caused a reduction in fertility indicates that the posterior pituitary hormones may have a direct effect on the sperm. This is further indicated by the results of Hughes and Parker (1970) who reported that addition of oxytocin to semen reduced sperm motility *in vitro* as well as the fertility of eggs from hens inseminated with it.

As already pointed out the egg spends about 20 hours in the uterus and during the final 17 hours shell material is deposited at a fairly uniform rate (Romanoff and Romanoff, 1949), but fertility is impaired only if insemination occurs during the last three hours of shell formation (Johnston and Parker, 1970; Hughes, 1970). Thus, if the hard-shelled egg *per se* does contribute to the reduction of fertility its effect is exerted through some factor peculiar to the last three hours of its formation. Bradfield (1951) reported that until the last one or two hours prior to oviposition the egg lies with its pointed end toward the posterior of the hen, then the egg undergoes a 180^{o} rotation so that the blunt end is toward the posterior of the hen. This change in position could make the egg a more complete block to sperm passage causing them to be retained in the vagina where the environment may not be as favorable to sperm as that in the more anterior regions of the oviduct.

The results of Hughes and Parker (1970) provide further evidence that the position of the hard-shelled egg in the oviduct one to

three hours prior to laying may be important. They inseminated hens that had hard-shelled eggs in their uteri at night (9:30 p. m.) and then induced the hens to lay prematurely by injecting them with oxytocin. Despite the presence of a hard-shelled egg in the uterus at the time of insemination and the laying of the egg shortly after insemination, fertility was not reduced as much as when hens lay naturally after insemination.

Epinephrine has been shown to inhibit the action of oxytocin on the uterus of the cow (VanDemark and Hays, 1951; Hays and VanDemark, 1953a). Should epinephrine inhibit the action of oxytocin on the avian uterus it would seem conceivable that it might overcome the adverse effect of oxytocin on fertility. Fertility tended to increase when hens without hard-shelled eggs in their uteri were given subcutaneous injections of epinephrine alone without oxytocin 20 minutes before insemination. But when "empty" hens were injected with oxytocin immediately after insemination the prior epinephrine injections did not counteract the adverse effect of oxytocin on fertility.

Since the epinephrine was given subcutaneously 20 minutes before the intravenous injection of oxytocin it may not have been absorbed rapidly enough to counteract the action of the oxytocin. One might speculate that intravenous injections of epinephrine might have given different results. Since such injections prior to insemination of hens with hard-shelled eggs in their uteri did not overcome the

fertility-reducing effect of laying shortly after insemination it is doubtful that intravenous injections of epinephrine would have counteracted the adverse effect of oxytocin on fertility. Based upon the results of these investigations it appears that epinephrine has no effect in counteracting oxytocin in hens or in overcoming the fertility-reducing effect of a hard-shelled egg in the uterus.

Subcutaneous injections of epinephrine in hens without hard-shelled eggs in their uteri significantly reduced egg production; whereas, intravenous injections of epinephrine in hens with hard-shelled eggs in their uteri had no effect on egg production. No definite explanation can be given for these differences in rates of lay following epinephrine injections, but the difference may be related to the fact that the two groups of hens were in different stages of egg formation or to the fact that the hormone was administered in different ways. Subcutaneous and intramuscular injections of epinephrine have been reported to have different effects on rate of egg production in chickens (Sykes, 1955b; Hawes, 1962). Perhaps future experiments involving the use of various methods of hormone administration at different stages of egg formation will elucidate further the role of epinephrine as related to fertility and rate of egg production.

The observation that injecting male chickens with oxytocin shortly before manual ejaculation caused a decrease in semen volume and had little effect on concentration and total sperm per ejaculate is

contrary to the findings in mammals. Oxytocin injections increased semen volume in rabbits (Kihlstrom and Melin, 1963) and increased semen volume, concentration and number of sperm per ejaculate in rams and bulls (Milovanov, Bereznev and Gorohov, 1962; Knight and Lindsay, 1970). The fact that male chickens do not have counterparts to prostate, Cowper's glands and vesicular glands might lead one to speculate that secretions from these organs in the mammalian males may account for the difference between the response of chicken males and mammalian males to oxytocin injections. However, since the oxytocin injections in mammalian males caused an increase in sperm concentration and total number of sperm per ejaculate it would seem that oxytocin acts mainly by increasing the number of sperm rather than by increasing secretions of the accessory glands. Bereznev (1964) reported that oxytocin injections increased smooth muscle contractions of testis, epididymis and vas deferens and also increased sperm accumulations in the ampulla of bulls. Therefore, it appears that the avian testis, epididymis and vas deferens do not respond to oxytocin in the same manner as do the corresponding mammalian organs.

VI. SUMMARY AND CONCLUSIONS

The primary objective of this study was to determine whether the presence of the posterior pituitary hormones that initiate oviposition was related to the reduced fertility following insemination shortly before or after oviposition. Injecting hens with the posterior pituitary hormones, oxytocin and arginine vasopressin, at various time intervals before or after insemination caused a pattern of fertility reduction similar to, but not as great as, that observed when normal oviposition occurred shortly before or after insemination.

The effect of oxytocin injections on semen production in cockerels also was studied. Contrary to results with some mammals, injecting cockerels with oxytocin shortly before manual ejaculation did not increase semen volume or number of sperm per ejaculate.

The following conclusions are drawn from this study.

1. Injecting hens with 3 or 5 I. U. of oxytocin immediately before insemination caused a significant reduction in fertility of eggs laid two to nine days following insemination. Oxytocin injections given 30 minutes before insemination caused a slight reduction in fertility and those given one hour, two hours or five hours before insemination had no effect.
2. Duration of fertility was reduced significantly when hens were injected with 5 I. U. of oxytocin immediately before

insemination, but was not affected when the injections were given 30 minutes, one hour, two hours or five hours before insemination. In one trial the reduction in duration of fertility was mainly due to infertile hens--those hens that were fertile exhibiting a normal duration of fertility--while in a second trial the reduction could not be attributed to infertile hens.

3. Injecting hens with 5 I. U. of oxytocin immediately or 30 minutes after insemination caused a significant reduction in fertility. Smaller and nonsignificant reductions in fertility were observed when the oxytocin injections were given one or two hours after insemination; and injections given five hours after insemination had no effect.

4. Injecting hens with 3 I. U. of arginine vasopressin immediately after insemination caused a significant reduction in fertility in one trial and a nonsignificant reduction in a second trial. When the injections were given 30 minutes and one hour after insemination small and nonsignificant reductions in fertility were observed in one trial and in another trial there was no difference. The shorter the time interval between inseminations and vasopressin injections the greater was the reduction in fertility.

5. Injecting hens intravenously with a saline solution containing

2.5 I. U. of oxytocin and 1.5 I. U. of arginine vasopressin immediately or 30 minutes after insemination caused significant reductions in fertility. Injections of this mixture one hour after insemination had no significant effect. The reduction in fertility caused by intravenous injections of the mixture of the two hormones immediately after insemination tended to be greater than that caused by injecting either 2.5 or 5.0 I. U. of oxytocin; however the differences between the three treatments were not statistically significant.

6. Single intravenous injections of oxytocin in hens one or two hours after insemination caused as great a reduction in fertility as when the intravenous injectipns were preceded by two or three subcutaneous injections of oxytocin.

7. Both intravenous and subcutaneous injections of 3 I. U. of oxytocin in hens immediately after insemination caused significant reductions in fertility, those caused by intravenous injections being greater.

8. Dilution of semen with oxytocin (2 I. U. /ml semen) or with epinephrine (0.125 mg/ml semen) caused small and non-significant reductions in fertility.

9. Subcutaneous injections of 1 mg of epinephrine in hens 20 minutes before insemination had a tendency to increase fertility when given alone, but were not effective in

counteracting the adverse effect on fertility caused by intravenous injections of 5 I. U. of oxytocin given immediately after insemination.

10. Intravenous injection of hens with 0. 5 mg of epinephrine 2 to 20 minutes before insemination or the addition of epinephrine to semen (0. 125 mg/ml semen) did not overcome the reduction in fertility related to oviposition shortly after the time of insemination.
11. Egg production was reduced when epinephrine injections were given subcutaneously but was not affected when the injections were given intravenously.
12. Injecting hens with oxytocin or epinephrine had no significant effect on hatchability.
13. Injecting cockerels with oxytocin before manual ejaculation usually caused a decrease in semen volume. Concentration of sperm showed a tendency to increase but the total number of sperm per ejaculate was not significantly affected by the oxytocin injections. The oxytocin injections had no effect on the fertilizing capacity of the semen or hatchability of the fertile eggs.

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APPENDIX

Table 1. Oviducal regions, their function and the time spent in each by the developing egg (Romanoff and Romanoff, 1949).

Oviducal region	Function	Duration of stay	
		hours	minutes
Infundibulum	Receives ovum and fertilization site		20
Magnum	Secretion of albumen	3	0
Isthmus	Formation of shell membranes	1	10
Uterus	Deposition of shell, shell pigment and thin albumen	19	0
Vagina	Passage from uterus to cloaca	Briefly	

Table 2. Effect of intravenous injections of oxytocin or a saline solution containing 0.25% acetic acid and 0.50% chlorobutanol immediately before insemination on fertility and hatchability. [1]

Group	Treatment	Hens (no.)	Eggs (no.)	Average fertility of hens (2-9 days) (%)	Average hatchability of hens (2-9 days) (%)
1	No injection (control)	19	104	97.8	89.5
2	5 I.U. of oxytocin	19	106	54.5**	89.2
3	Acetic-acid-chlorobutanol solution (0.25 ml)	18	105	96.9	94.1

[1] All hens laid at least four hours prior to treatment.

** Difference from treatments 1 and 3 is significant at the $P < .01$ level.

Table 3. Effect of various amounts of oxytocin injected intravenously immediately before insemination on fertility and hatchability. [1]

Treatment	Average fertility of hens						Average hatchability of hens (2-17 days) (%)
	2-9 days			10-17 days			
	Hens (no.)	Eggs (no.)	%	Hens (no.)	Eggs (no.)	%	
No injection (control)	19	92	90.8	18	93	32.8	77.6
1 I. U. of oxytocin	20	98	74.4	19	86	21.3	80.6
3 I. U. of oxytocin	19	100	46.7**	19	96	25.5	84.8
5 I. U. of oxytocin	19	89	42.2**	19	92	18.8	92.7

[1] All hens laid at least two hours prior to treatment.

** Difference from controls is significant at the $P < .01$ level.

Table 4. Effect of various amounts of oxytocin injected intravenously immediately before insemination on duration of fertility. [1]

	Treatment	Average duration of fertility (days)[2]	
		Infertile hens included	Infertile hens excluded
1	No injection (control)	10.8	10.8
2	1 I. U. of oxytocin	8.8	9.2
3	3 I. U. of oxytocin	8.2	9.8
4	5 I. U. of oxytocin	5.7**	10.9

[1] All hens laid at least two hours prior to treatment.

[2] Duration was measured as the last day an individual hen laid a fertile egg during a period of 17 days following insemination.

** Difference from controls is significant at the $P < .01$ level.

Table 5. Effect of intravenous injections of 5 I. U. of oxytocin at various times before insemination on fertility and hatchability. [1]

Group	Time of injection before insemination	Average fertility of hens						Average hatchability of hens (2-17 days) (%)
		2-9 days			10-17 days			
		Hens (no.)	Eggs (no.)	%	Hens (no.)	Eggs (no.)	%	
1	No injection (control)	15	103	83.6	15	105	26.8	86.6
2	Immediate	15	107	26.9**	15	99	2.9**	95.8
3	30 minutes	15	102	77.9	15	110	21.7	84.9
4	1 hour	15	97	87.2	15	109	21.6	78.8
5	2 hours	15	96	95.4	14	98	35.6	88.1
6	5 hours	15	102	90.1	15	99	37.7	77.9

[1] All hens laid at least two hours prior to treatment.

** Difference from all other treatments is significant at the $P < .01$ level.

Table 6. Effect of intravenous injections of 5 I. U. of oxytocin at various times before insemination on duration of fertility.[1]

Group	Time of injection before insemination	Average duration of fertility (days)[2]	
		Infertile hens included	Infertile hens excluded
1	No injection (control)	11.5	11.5
2	Immediate	4.2**	7.9*
3	30 minutes	11.3	11.3
4	1 hour	11.3	11.3
5	2 hours	12.8	12.8
6	5 hours	11.9	11.9

[1] All hens laid at least two hours prior to treatment.

[2] Duration was measured as the last day an individual hen laid a fertile egg during a period of 17 days following insemination.

* Difference from controls is significant at the $P < .05$ level.

** Difference from controls is significant at the $P < .01$ level.

Table 7. Effect of intravenous injection of 5 I. U. of oxytocin at various times after insemination on fertility and hatchability. [1]

Group	Time of injection after insemination	Hens (no.)	Eggs (no.)	Average fertility of hens (2-9 days) (%)	Average hatchability of hens (2-9 days) (%)
1	No injection (control)	20	121	91.3	86.9
2	Immediate	19	106	57.7**	77.9
3	30 minutes	19	104	64.3**	76.0
4	1 hour	20	122	78.3	94.6
5	2 hours	19	113	85.6	88.9
6	5 hours	20	122	94.6	86.4

[1] All hens laid at least two hours prior to treatment.

** Difference from controls is significant at the $P < .01$ level.

Table 8. Effect of intravenous injections of 3 I. U. of arginine vasopressin at various times after insemination on fertility and hatchability. [1]

Group	Time of injection after insemination	Hens (no.)	Eggs (no.)	Average fertility of hens (2-9 days) (%)	Average hatchability of hens (2-9 days) (%)
1	No injection (control)	15	100	90.8	93.6
2	Immediate	15	97	75.0	94.0
3	30 minutes	15	99	78.2	90.9
4	1 hour	15	97	84.2	89.1

[1] All hens laid at least two hours prior to treatment.

Table 9. Effect of intravenous injections of a saline solution containing 2.5 I. U. of oxytocin and 1.5 I. U. of vasopressin at various times after insemination on fertility and hatchability. [1]

Treatment	Time of injection after insemination	Hens (no.)	Eggs (no.)	Average fertility of hens (2-9 days) (%)	Average hatchability of hens (2-9 days) (%)
No injection (control)		15	98	95.5	89.0
0.3 ml of saline (control)	Immediate	10	73	84.6	87.5
oxytocin-vasopressin	Immediate	15	96	46.9**	92.2
oxytocin-vasopressin	30 minutes	15	97	71.4*	95.5
oxytocin-vasopressin	1 hour	15	101	87.5	92.3

[1] All hens laid at least two hours prior to treatment.

* Difference from uninjected controls is significant at the $P < .05$ level.

** Difference from uninjected and saline injected controls is significant at the $P < .01$ level.

Table 10. Effect of oxytocin, vasopressin and oxytocin-vasopressin mixture injected intravenously after insemination on fertility and hatchability.[1]

	Treatment	Time of injection after insemination	Hens (no.)	Eggs (no.)	Average fertility of hens (2-9 days) (%)	Average hatchability of hens (2-9 days) (%)
1	0.3 ml of saline (control)	Immediate	15	87	94.1	82.5
2	3 I.U. of vasopressin	Immediate	15	93	65.6*	84.6
3	3 I.U. of vasopressin	30 minutes	15	88	95.3	96.0
4	3 I.U. of vasopressin	1 hour	15	90	93.4	92.1
5	2.5 I.U. of oxytocin - 1.5 I.U. of vasopressin	Immediate	15	88	32.0**	95.9
6	2.5 I.U. of oxytocin	Immediate	15	92	51.8**	79.1
7	5.0 I.U. of oxytocin	Immediate	15	91	40.0**	91.0

[1] All hens laid at least three hours prior to treatment.

* Difference from controls is significant at the $P < .05$ level.

** Difference from controls is significant at the $P < .01$ level.

Table 11. Effect of single and multiple injections of oxytocin after insemination on fertility and hatchability. [1]

	Treatment	Time of injection after A. I.	Hens (no.)	Eggs (no.)	Average fertility of hens (2-9 days) (%)	Average hatchability of hens (2-9 days) (%)
1	Saline, 0.3 ml, iv[2] (control)	Immediate	12	77	94.8	85.6
2	Oxytocin, 3 I. U., iv	Immediate	12	74	47.7**	86.1
3	Oxytocin, 3 I. U., sc	Immediate	12	77	66.0*	84.2
4	Oxytocin, 1 I. U., sc Oxytocin, 1 I. U., sc Oxytocin, 3 I. U., iv	Immediate 30 minutes 1 hour	11	63	72.4	75.6
5	Oxytocin, 1 I. U., sc Oxytocin, 1 I. U., sc Oxytocin, 1 I. U., sc Oxytocin, 3 I. U., iv	Immediate 30 minutes 1 hour 2 hours	12	73	84.6	94.2

[1] All hens laid at least three hours prior to treatment.

[2] iv - intravenous injection; sc - subcutaneous injection.

* Difference from controls is significant at the $P < .05$ level.

** Difference from controls is significant at the $P < .01$ level.

Table 12. Effect of diluting semen with oxytocin, epinephrine or an acetic acid-chlorobutanol solution on fertility and hatchability. [1]

Group	Semen dilution (per ml of semen)	Hens (no.)	Eggs (no.)	Average fertility of hens (2-9 days) (%)	Average hatchability of hens (2-9 days) (%)
1	Undiluted semen	23	117	96.8	93.0
2	0.1 ml (2 I.U.) of oxytocin	20	104	78.6	96.7
3	0.125 ml (0.125 mg) of epinephrine	19	97	85.0	84.7
4	0.1 ml of a saline solution containing 0.25% acetic acid and 0.50% chlorobutanol	18	94	92.7	80.3

[1] All hens had laid at least four hours prior to treatment.

Table 13. Counteracting the adverse effect of oxytocin on fertility by epinephrine injections and the effect of oxytocin and epinephrine on hatchability and egg production. [1]

	Treatment	Hens (no.)	Eggs (no.)	Average fertility of hens (2-9 days) (%)	Average hatchability of hens (2-9 days) (%)	Egg production (hen days) (%)
1	Intravenous injection of 0.5 ml of saline immediately after insemination (control)	15	79	84.1	93.8	65.8
2	Intravenous injection of 5 I. U. of oxytocin immediately after insemination	15	87	40.7**	98.2	72.5
3	Subcutaneous injection of 1 mg of epinephrine 20 minutes before insemination	14	56	100.0	94.2	46.7*
4	Subcutaneous injection of 1 mg of epinephrine 20 minutes before insemination and an intravenous injection of 5 I. U. of oxytocin immediately after insemination	13	55	44.2**	95.0	45.8*

[1] All hens laid at least three hours prior to treatment.

*Difference from controls is significant at the $P < .05$ level.

**Difference from controls is significant at the $P < .01$ level.

Table 14. Effect of epinephrine on fertility, hatchability and egg production of hens inseminated when a hard-shelled egg was present in the uterus.

	Treatment	Hens (no.)	Eggs (no.)	Average fertility of hens (2-9 days) (%)	Average hatchability of hens (2-9 days) (%)	Egg production (hen days) (%)
1	Hens inseminated with undiluted semen (control)	19	97	65.2	81.3	63.8
2	Intravenous injection of 0.5 mg (0.5 ml) of epinephrine 2 to 20 minutes before insemination	16	86	40.2	97.5	67.2
3	Hens inseminated with semen diluted with 0.125 mg (0.125 ml) of epinephrine per ml	16	88	38.5	74.7	68.8

Table 15. Effect of intravenous injection of White Leghorn and New Hampshire cockerels with 1 and 5 I. U. of oxytocin before ejaculation on semen volume, concentration of sperm and total number of sperm per ejaculate.

Treatment[1]	Average semen volume (ml)	Average concentration of sperm		Average number of sperm per ejaculate (billions)
		per mm^3 of semen (millions)	packed sperm volume (%)	
No injection (control)	0.85	2.89	7.6	3.02
0.5 ml of saline	0.82	2.15	6.9	1.93
1 I. U. of oxytocin	0.65	2.46	8.9	1.64
5 I. U. of oxytocin	0.46**	2.84	10.4*	1.42

[1]Ten males per treatment.

*Difference from controls is significant at the $P < .05$ level.

**Difference from controls is significant at the $P < .01$ level.

Table 16. Effect of intravenous injection of White Leghorn and New Hampshire cockerels with 1 and 5 I. U. of oxytocin before ejaculation on fertilizing capacity of semen and hatchability of fertilized eggs.

Treatment[1]	Hens (no.)	Eggs (no.)	Average fertility of males (2-9 days) (%)	Average hatchability of males (2-9 days) (%)
No injection (control)	30	191	89.8	84.5
0.5 ml of saline	30	187	91.2	91.4
1 I. U. of oxytocin	29	189	83.2	84.9
5 I. U. of oxytocin	28	181	87.6	80.8

[1]Ten males per treatment.

Table 17. Effect on volume and concentration of sperm of intravenous injection of untrained White Leghorn and Cornish cockerels with 2.5 I.U. of oxytocin before manual ejaculation.

Treatment	Average semen volume (ml)	Average concentration of sperm (packed sperm volume) (%)
	Leghorn males[1]	
No injection (control)	0.53	9.2
2.5 I.U. of oxytocin	0.32*	13.8*
	Cornish males[2]	
First trial		
No injection (control)	0.49	9.7
2.5 I.U. of oxytocin	0.46	10.3
Second trial		
No injection (control)	0.41	7.3
2.5 I.U. of oxytocin	0.49	9.7

[1] Six males per treatment.

[2] Three males per treatment.

* Difference from controls is significant at the $P < .05$ level.

Table 18. Effect of intravenous injection of White Leghorn and Cornish cockerels with 2.5 I. U. of oxytocin before ejaculation on fertilizing capacity of semen and hatchability of fertilized eggs.

Treatment	Hens (no.)	Eggs (no.)	Average fertility of males (2-10 days)[1] (%)	Average hatchability of males (2-10 days)[1] (%)
		Leghorn males[2]		
No injection (control)	12	77	90.0	84.5
2.5 I. U. of oxytocin	12	83	83.9	75.8
		Cornish males[3]		
First trial				
No injection (control)	6	40	83.3	84.9
2.5 I. U. of oxytocin	6	43	88.4	94.6
Second trial				
No injection (control)	9	40	55.1	46.7
2.5 I. U. of oxytocin	9	38	75.9	69.3

[1] In the second trial with Cornish males fertility and hatchability was based on eggs laid two to nine days following insemination.

[2] Six males per treatment.

[3] Three males per treatment.

Table 19. Effect of intravenous injection of White Leghorn cockerels with 0.25, 1 and 5 I. U. of oxytocin before ejaculation on semen volume and concentration of sperm.

Treatment[1]	Average semen volume (ml)	Average concentration of sperm (packed sperm volume) (%)
0.5 ml of saline (control)	0.76	11.2
0.25 I. U. of oxytocin	0.54	12.6
1.0 I. U. of oxytocin	0.62	15.0
5.0 I. U. of oxytocin	0.55	17.2

[1]Five males per treatment.

Table 20. Effect of intravenous injection of White Leghorn cockerels with 0.25, 1 and 5 I. U. of oxytocin before ejaculation on fertilizing capacity of semen and hatchability of fertilized eggs.

Treatment[1]	Hens (no.)	Eggs (no.)	Average fertility of males (2-9 days) (%)	Average hatchability of males (2-9 days) (%)
0.5 ml of saline (control)	14	81	94.0	86.6
0.25 I. U. of oxytocin	15	75	89.8	66.6*
1.0 I. U. of oxytocin	13	74	93.4	81.4
5.0 I. U. of oxytocin	14	87	89.5	90.5

[1]Five males per treatment.

*Difference from 5 I. U. of oxytocin is significant at the $P < .05$ level.

Table 21. Effect of intravenous injection of White Leghorn cockerels with 0.05, 0.25 and 1.0 I.U. of oxytocin before ejaculation on semen volume, concentration of sperm and total number of sperm per ejaculate.

Treatment[1]	Average semen volume (ml)	Average concentration of sperm per mm^3 of semen (millions)	Average concentration of sperm packed sperm volume (%)	Average number of sperm per ejaculate (billions)
0.25 ml of saline (control)	0.90	3.68	11.3	3.34
0.05 I.U. of oxytocin	0.97	3.64	11.3	3.45
0.25 I.U. of oxytocin	0.73	4.33	12.3	3.25
1.00 I.U. of oxytocin	0.69*	4.52	13.2	3.06

[1]Six males per treatment.

*Difference from controls is significant at the $P < .05$ level.

Table 22. Effect of intravenous injection of White Leghorn cockerels with 0.05, 0.25 and 1.0 I.U. of oxytocin before ejaculation on fertilizing capacity of semen and hatchability of fertilized eggs.

Treatment[1]	Hens (no.)	Eggs (no.)	Average fertility of males (2-9 days) (%)	Average hatchability of males (2-9 days) (%)
0.25 ml of saline (control)	18	113	92.4	77.4
0.05 I.U. of oxytocin	17	104	91.2	83.5
0.25 I.U. of oxytocin	17	95	90.7	73.6
1.00 I.U. of oxytocin	16	99	75.3	60.6

[1]Six males per treatment.