

**INEFFECTIVENESS OF ESTROGEN-PROGESTERONE HORMONE
TREATMENT IN EVOKING UDDER GROWTH
IN BUFFALO HEIFER**

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SUMMARY

Six Egyptian buffalo heifers were subcutaneously injected daily with 100 mg. progesterone and 100 µg. estradiol. Three out of them were cycling and were given the hormone injections for 90 days. No response was noticed neither in the udder glandular tissue nor in the teats. The size of the ovaries, however, decreased and no structures were detected on their surfaces. A fourth heifer was 60 days pregnant when subjected to the hormone treatment for 37 days which apparently caused abortion between the second and the third week of injection period. No signs of udder growth were observed during such period as well as during a period of 60 days following treatment. Another 150 days pregnant heifer was injected for 120 days by the same hormonal combination. This heifer delivered a calf 270 days old and no milk was yielded after calving. During the injection period and for 30 weeks post treatment no signs of estrus were observed and no significant external changes were observed neither in the udder size nor in the glandular tissue. The sixth heifer was 161 days pregnant when subjected to 120 days hormone treatment; it calved after 291 days producing only 213 kgs of milk during 168 day lactation period. The udder growth did not respond to such treatment and non-significant ovarian structures or heat symptoms were observed throughout the 31 weeks after the last injection.

INTRODUCTION

Researches in physiology of lactation are usually directed towards the finding of the hormonal mechanisms controlling the development of the mammary gland itself, and into the factors influencing the secretion and ejection of milk from glands so formed. Studies on the hormonal induction of udder growth and lactation in goats (Minxner and Turner, 43, Cowie *et al.*, 52) and calves (Sykes and Wrenn, 51) show that the secretory tissue of the udders developed by treatment with estrogen and progesterone is histologically more normal than that of those developed by Folley *et al.* (40). The ratio of these two hormones for the optimal growth of labule alveolar system in mice, rats and dogs is 1 estrogen to 1000 or more of

progesterone (Elliot and Turner, 53, Yamada *et al.*, 54, in mice), (Elliot and Turner, 53, in rats) and (Trentin *et al.*, '52, in dogs). In dairy calves, Yamamoto and Turner, 55, Williams *et al.*, 55, Turner *et al.*, 56, Williams and Turner, 59, and Turner, '59, injected 100 mg. progesterone and 100 μ g. estrogen daily for a period of 180 days for determining the optimum ratio and dose of the two ovarian hormones and their synergism for growth of the udders of heifers. Similar investigations, unfortunately, have not been carried out in buffaloes; it was therefore thought desirable to make some observations on the effectiveness of such ovarian hormones in evoking udder growth in buffalo heifers. It seemed important to start with clarifying the physiological causes of the common problem of the mature buffalo heifer that usually shows poorly developed udder even after calving. We have, accordingly, carried out two experiments on the effects of the administration of combined estrogen and progesterone in evoking the growth of the glandular tissue in the udders of pregnant and nonpregnant Egyptian buffalo heifers.

MATERIALS AND METHODS

Animals : Six Egyptian buffalo heifers belonging to Ain shams Faculty of Agriculture Experimental Station, were divided into two groups and subjected to two treatments :

Group I. Comprised three heifers 20-24 months old (Nos. 180, 188 and 255). These heifers were selected to be used in this treatment after being checked twice daily with an active bull and showed at least one complete estrous cycle. Meanwhile, they were examined by rectal palpation prior and allthrough treatment period in order to determine the condition of the reproductive organs.

Group II. included three pregnant heifers about 4 years old. Two animals (Nos. 214 and 223) were 150 and 161 days pregnant and the third one (No.95) was 60 days pregnant Average gestation period in buffaloes 316;6 according to Mohammed, 65).

Hormones : progesterone USP*, (P) and Estradiol USP** (E) was used and dissolved in maize oil to make a concentration of 50 mg. (P) and 50 μ g. (E) per cc. The solution in oil was prepared by dissolving the crystalline progesterone (or estradiol) in pure ether and then the maize oil was added gradually. The flasks containing the mixture were then put into an automatic shaker for 8 hrs. then refluxed to evaporate the ether.

Experimental procedure : One daily injection of 100 mg (P) and another daily injection of 100 μ g. (E) were subcutaneously injected in the neck region.

* Furnished by Mann Research Laboratories Inc., New York.

** Furnished by Nutritional Biochemicals Corporation, Cleveland Ohio.

In the first experiment, it was planned to inject the two ovarian hormones in 3 cycling heifers for a period of 90 days with the assumption that this treatment period might be sufficient to stimulate the growth of the udder glandular tissue prior to pregnancy, so that when pregnancy insues a considerable udder development might be attained either spontaneously or with the help of additional hormone treatment during the second half of gestation.

It was kept in mind the possibility that such heifers injected with hormones may not conceive readily and therefore the second experiment was also planned. In the second experiment, a heifer 60 days pregnant as well as two other heifers 150 and 161 days pregnant were used in view of stimulating the udder development in early and advanced pregnant heifers. With regard to the first heifer, the 60 days pregnancy was considered adequate for the establishment and attachment of placenta. It was intended to subject this heifer to the hormone treatment for 3 months since in normal pregnancy the growth of the lobule-alveolar system takes place during the first two thirds of gestation period (Turner, 59). This heifer, however, received the hormone treatment for only 37 days then stopped when the interruption of pregnancy was detected between the second and third weeks of the injection period. The two heifers in advanced pregnancy were injected with the combined hormones for 4 months to evoke the lobule-alveolar growth in the second half of pregnancy.

Index of Udder Growth : Two considerations has been taken in account in deciding the required index for udder growth. First, the value of the animal precludes a direct estimation of the extent of mammary gland growth which requires sacrificing the animals at the end of the experimental growth stimulation period. Second, the maximum daily milk yield when considered as an index for udder development, is influenced by several factors including the condition of the animal, hormonal status and the extent of glandular growth. Furthermore, the initiation of lactation by means of estrogenic hormones is still liable to some criticism (Turner, 59). On the other hand much attention has been paid to morphological studies of the artificially developed udder by methods specially developed for the purpose.

The changes in the udder were determined biweekly using the palpation method adopted by Swett *et al* ('55). The udders in the later stage of glandular development were measured using an ordinary standard metric ribbon for obtaining the whole length of each half and the maximum width and depth of quarters. The length and the maximum diameter of teats were measured using a steel Caliper. In the nonpregnant heifers, the changes in the genital organs were determined weekly by rectal palapatation to make digital measurements of uterus and ovaries.

RESULTS AND DISCUSSION

EXPERIMENT I.

I.—*Response of the Mammary Glands to Hormone Treatment.*

No changes were observed in the udder shape, size, position, firmness or the amount of fat deposition. In no case there was any evidence of mammary

gland response to the doses employed as all udders in the treated group did not differ from the control group. The teats did not show any significant change neither in their length and diameter nor in their placement during the treatment period. The failure of the combined estradiol and progesterone treatment, under the conditions of this experiment in producing significant mammary growth can be clarified according to the following considerations:

(1) The slow development of the udders in buffalo heifers seems to be innate in this species, as no significant development was detected in the udders of the control heifers. It is a known fact that the interaction between estrogen and progesterone with respect to mammary gland growth varies markedly in the different zoological species (Courrier, 50). This is by no means curious as species differ in the age of attaining their body and sexual maturity which is correlated with the degree of development in their udders. Among the same species it is observed that individuals differ in size and type of tissue present in the udder which could be attributed to both inherited factors as well as other factors including the development in the functional activity of the endocrine glands which in turn could be affected by many factors (Jacobsohn, 61).

(2) The udder may be the least sensitive reproductive part to hormone application in the buffalo, since the udder of the experimental heifers showed no response to hormone treatment while the uterus and ovaries responded to it significantly. Courrier ('50), projected the differential sensitivity of the receptor organs in hormonal interactions.

(3) Although the heifers used in this study, were all of relatively good size yet their reproductive organs were relatively small. El-Sheikh ('62) noticed that the low level of nutrition affects markedly the development of the reproductive organs in the buffalo heifers. According to Leatham ('61), definite effects of nutrition have been observed on mammary growth, the steroid stimulation of the mammary gland was found to be influenced by nutritional factors. Treutin and Turner ('41), showed that as food intake decreased, the amount of estradiol required to produce a minimal duct growth was proportionately increased. On the other hand inanition did reduce the content of hypophyseal lactogen in the rat, according to Meites and Reed ('49).

(4) It is possible that the failure of the udders of sexually mature heifers used, to respond to the hormone treatment is due to the lowered sensitivity of buffalo udders. Anterior pituitary hormones seem to be essential for the growth-promoting actions of estrogen and progesterone. The results of the present work agree well with those of Sykes and Wrenn ('50). They used the dairy heifers to study the type of development attained by stilbestrol or stilbestrol plus progesterone alone and in combination with pituitary extracts. They found that the failure of steroid hormones to produce alveolar development comparable to that produced by the combined action of steroids and pituitary extracts could have been a result of inadequate sensitization or maturation of the rudimentary mammary tissues by pituitary hormones.

Thereupon, they concluded that estrogen or estrogen plus progesterone will produce alveolar development at any stage, but a period of sensitization by pituitary hormones is necessary before their effects may be realized. A situation somewhat analogous to this can be visualized as occurring during normal pregnancy in cattle. Until the third or fourth month of pregnancy the udders of heifers consist solely of an extensive system of ducts (Sykes and Wrenn, '5p). This rather prolonged period may be a period of extension and maturation of udder rudimentary tissues primarily under the influence of pituitary hormones since the amounts of steroid hormones secreted during this interval are relatively low. The rapid development of alveoli during the latter two-thirds of pregnancy could be a result of the previous maturation combined with the increased secretion of steroid hormones which occurs during pregnancy (Sykes and Wrenn, '50).

The foregoing is in line with what has been concluded by Folley ('61), as the pituitary hormones cooperation with ovarian hormones seem necessary for the experimental production of normal mammary growth similar to that occurring during pregnancy. Early, Petersen ('48) cited that there is a considerable evidence that prolactin plays an important role in mammary growth. Recently, such role has been greatly clarified through studying the effect of suckling on mammary development, while suckling elicits prolactin release from the anterior pituitary in several species (Grosvenor and Turner '57a,b,c, Reece and Turner, '37, Seyle *et al.*, '34, Seyle and McKeown, '34) it promotes mammary development and lactation (Seyle, '34, Seyle *et al.*, '34, Seyle and McKeown, '34a,b).

(5) An assumption of antagonism between the estradiol and progesterone used in the present experiment could be ruled out for at least two considerations. First, the ratio of estradiol to progesterone (1:1000) as used has appeared to be the optimal ratio for full mammary gland growth in cattle and many laboratory animals as previously shown. Second, the dose levels of the hormones injected could not be considered so high to justify the assumption of antagonism. On one hand, Williams and Turner ('61) studied the effect of increased levels of ovarian hormones on the experimental induction of growth of the cow's udder. They found that daily injection of 200 mg. of progesterone with 200 ug. estradiol benzoate for 180 days apparently stimulated mammary gland growth in no way greater than 100 mg. of progesterone and 100 ug. of estradiol benzoate injected daily for 180 days. On the other hand the doses used in the present experiment were capable to produce significant growth in the uterus. The ovarian hormones in their action upon uterus, have a nearly parallel effect on the associated phenomenon of mammary growth (Folley and Malpress, '48).

II.—Response of the Ovaries to the Hormone Treatment:

The results obtained from rectal palpation showed that no ovarian structures were detected during the treatment period in the treated heifers, such heifers had less average ovarian size after hormone injections than before,

while the control heifers showed normal activity during the period of experiment (table I). Significant differences ($P < 0.01$) between the ovarian size before and after treatment as well as between the treated and the control heifers were observed. Through the period of the injection of the ovarian hormones, oestrous cycles were inhibited. The foregoing results concerning the ovarian activity may be explained according to Ulberg *et al* (1951) who assumed that progesterone has a blocking effect "upon the action of gonadotrophic complex (perhaps primarily upon the LH). Béla Flerko' ('63) reviewed experimental results, that appear to support the hypothesis of a "nervous feed back control" by which sex steroids exert their action upon gonadotropin secretion. On the other hand he discussed experimental data supporting the hypothesis that in the rat nervous elements of the anterior hypothalamus are indispensable in the "feed back mechanism" by which gonadotropin release is inhibited already by a slight (physiologic) elevation of the sex steroid level in the blood. Thus it may seem reasonable to assume that progesterone injected in the present experiment was responsible for the suppression of both the follicular development and symptoms of heat through the injection interval.

Response of the Uterus to the Hormone Treatment :

The results obtained from rectal palpation in this experiment (table II) showed that there was a significant increase ($p < 0.05$) in the diameter of the uterine horns, such horns appeared to be normal in texture. The uterine horns of the control heifers, on the contrary, showed no significant increase in diameter. Turner ('49), stated that because of the synergistic action of estrogen and progesterone, a combination of the two hormones is more or less favourable for complete uterine differentiation than when either is given alone. Recently, Hissaw and Hissaw ('61), stated that the growth of the endometrium is greater when the two ovarian hormones are given together due to their synergistic interaction. They added that the progestational development of the endometrium, when both hormones were given, passes through the same stages as those following the injection of progesterone alone. The endometrium, however, is considerable thicker than a comparable dose of progesterone is given alone.

EXPERIMENT 2.

In this experiment three pregnant buffalo heifers (60.50, 161 days pregnant) were injected with the two ovarian hormones used in the first experiment to study the effect of combined estrogen-progesterone hormones on mammary gland growth during early and advanced stages of pregnancy. Pregnancy was diagnosed prior to this experiment by rectal palpation.

One heifer (No. 95) received the hormone treatment starting on day 60 after conception and was continued daily for 37 days. The treatment caused the death of the embryo and its absorption between the second and the third week following the injection as detected by the tonus of the uterus and by the

TABLE 1.—AVERAGE OVARIAN SIZE* (mm³) BEFORE AND DURING TREATMENT

	Experimental Animals						Control Animals			
	255		180		188		178		173	
	Right ovary	Left ovary	Right ovary	Left ovary	Right ovary	Left ovary	Right ovary	Left ovary	Right ovary	Left ovary
30 days pretreatment interval.	3603.8	3003.4	3091.9	2489.1	2283.8	2856.0	2451.4	2428.3	2391.0	3023
90 days treatment interval	1892.5	1892.5	1574.8	1551.0	1300.0	1386.0	2338.3	3050.6	2376.0	2581

* Figures represent size produced by using the following equation :

$$\frac{4}{3} \cdot \frac{22}{7} \left(\frac{1}{2} \text{ length} \right) \left(\frac{1}{2} \text{ width} \right) \left(\frac{1}{2} \text{ height} \right).$$

TABLE 2.—AVERAGE DIAMETER (mm) OF UTERINE HORNS BEFORE AND DURING TREATMENT.

	Experimental Animals						Control Animals			
	255		180		188		178		173	
	Right horn	Left horn	Right horn	Left horn	Right horn	Left horn	Right horn	Left horn	Right horn	Left horn
30 days pretreatment interval.	18.0	16.7	20.6	22.3	16.3	17.0	20.5	21.0	20.0	18.0
90 days treatment interval	20.2	21.0	24.1	24.1	20.0	22.8	22.8	22.0	22.0	22.0

decrease in size of corpus luteum. Sixty one days after the last injection, the same heifer was observed in estrus again. No signs of udder growth were noticed neither during the treatment period nor during the 2 months post treatment.

Heifer No. 214 (150 days pregnant) was injected for a period of 120 days with the combined estradiol and progesterone hormones. This heifer delivered a male calf 270 days old. Following parturition, it was palpated weekly for determining the changes in the reproductive organs. On day one after parturition only small follicles 12 mm in diameter were detected. The same condition of lowered ovarian activity was noticed during the 30 weeks observation period after parturition. Moreover, no signs of estrus were noticed during the 30 weeks. No significant external differences in the size of the udder between treated and nontreated heifer were observed during the injection period or during the 30 weeks postpartum period. This heifer produced no milk.

The third heifer No. 223 (161 days pregnant) was injected with the same combination of hormones in the same doses for 120 days. It calved a male calf 291 days old, and produced only 123 kgs of milk in a lactation period of 168 days. This heifer was also observed for 30 weeks postpartum and only small follicles of about 12 mm. in diameter could be palpated. The heifer was checked for heat during the 30 weeks but no signs of heat were observed.

It is well known that the corpus luteum is the main source of progesterone hormone in the early stages of pregnancy, but, in advanced stages such hormone is mainly secreted by extraovarian sites, particularly, the placenta. It is possible that the hormone treatment used in this experiment inhibited the activity of the corpus luteum of pregnancy. Consequently, the inhibition of the corpus luteum at the early stages of pregnancy was evidently responsible for the death of the foetus and its absorption during the early stages of gestation. Such inhibition was not so detrimental when the treatment started in more advanced stage of pregnancy. Furthermore, the disturbance of gestation which occurred when treatment started on day 60 of gestation could be due to what was termed by Courrier ('50), "the Follicular abortion" which, as Courrier believes, is due to some antagonism between estradiol and progesterone injected. Courrier ('50), stated that estrogen is able to prevent the action of progesterone on the mucosa of the uterus in the mouse, rabbit, guinea pig, cat and rat. Although estrogen when injected alone in pseudopregnant and pregnant rabbits and rats (Bradbury, '61, Hammond Jr. and Robson, '51) would act as a luteotropic agent possibly through its effect on A.P. to release the secretion of luteotropic hormone maintaining C.L. hormone secretion yet when estrogen was injected with progesterone as in the present experiment in the ratio 1:1000 and in the doses injected were apparently not the optimal levels and doses that would be necessary for maintenance of pregnancy in the buffalo.

According to Folley ('61) and Jacobsohn ('61), it is well known now that the hormones of anterior pituitary, ovaries and adrenal cortex are the principle hormonal mechanisms regulating mammary gland growth. Jacobsohn ('61)

also stated that ovarian hormones exert some effect on the pituitary gland which in turn influence the mammary glands either directly or via target organs (ovaries, adrenals). Jacobsohn added that hormones produced by hypophyseal target organs can act in turn upon the anterior pituitary gland and alter its functional activity. Which regard to this situation further studies should be carried out to determine the possible effects of pituitary hormones when administered along with the ovarian hormones for evoking udder growth in buffalo heifer.

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عدم جدوى المعاملة بالأستروجين - بروجسترون في تنبيه نمو الضرع في عجلات الجاموس

الملخص

استعمل في هذا البحث ٦ عجلات جاموس كانت تحقن تحت الجلد يوميا بمقدار ١٠٠ مليجرام بروجسترون + ١٠٠ ميكروجرام استراديول وكان من بين هذه العجلات ثلاث يتكرر بها دورات الشبق عوملت بالهرمونات لمدة ٩٠ يوما ولم يحدث نتيجة الحقن بالهرمونات تغير ملموس سواء في النسيج الفدى بالضرع ولا بالحلمات ولكن لوحظ نقص في حجم المبايض وخلو المبيضين من الحويصلات الناضجة أو الأجسام الصفراء .

أما العجلة الرابعة فكانت حاملا لمدة ٦٠ يوما عند بدأ المعاملة بالهرمونات واستمرت المعاملة لمدة ٣٧ يوما وحدث على ما يبدو اجهاض نتيجة المعاملة بالهرمونات بين الأسبوع الثاني والثالث من الحقن بالهرمونات ولم يلاحظ نمو بالضرع خلال فترة المعاملة ولا في فترة ٦٠ يوم التالية لانتهاء المعاملة .

أما العجلة الخامسة فكانت حاملا لمدة ١٥٠ يوم عند بدأ المعاملة بالهرمونات واستمرت المعاملة لمدة ١٢٠ يوما ووضعت هذه العجلة عجلا بعد مدة حمل قدرها ٢٧٠ يوما فقط ولم تدر هذه العجلة لبنا بعد الوضع وفي هذه العجلة أيضا لم تشاهد علامات للشبق طول فترة المعاملة ولا بعد انتهاء المعاملة لمدة طولها ٣٠ اسبوعا كما لم تظهر تغيرات خارجية واضحة سواء في حجم الضرع ولا في النسيج الفدى .

أما العجلة السادسة فكانت حاملا لمدة ١٦١ يوما عند بدأ المعاملة التي استمرت لمدة ١٢٠ يوما متوالية وقد وضعت هذه العجلة بعد مدة حمل طولها ٢٩١ يوما فقط وكان مجموع ادرار هذه العجلة من اللبن بعد الوضع هو ٢١٣ كيلو جراما وذلك في مدة حليب طولها ١٦٨ يوما وهنا أيضا لم تحدث استجابة للمعاملة بالهرمونات في نمو الضرع كما لوحظ خلو المبيضين من التراكيب المبيضية (الحويصلات والأجسام الصفراء) ولم تظهر هذه العجلة علامات الشبق لمدة ٣١ أسبوعا تالية لآخر حقنة أعطيت من الهرمونات .