

## INFERTILITY IN MARES CAUSED BY OVARIAN DYSFUNCTION.

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### INTRODUCTION.

Many factors are concerned in the causation of infertility in equines, and abnormal ovarian function is generally regarded as the most important of all. Burkhardt (1948) considers that ovarian dysfunction is responsible for about 80 per cent of mares presented for treatment during the breeding season.

All previous research work indicates that abnormal functioning of the ovaries of the mare is due, not so much to infectious or pathological conditions of the genitalia, as to a disturbance in the mechanism which normally controls ovarian activity, that is, it is an upset of the delicate hormonal equilibrium which is essential for successful reproduction.

Imbalance of the gonadotrophic and oestrogenic hormones is usually manifested by aberrations in the oestrous cycle of the animal. In equines this cycle is regarded as seasonal, consisting of an active phase from spring to autumn during which breeding takes place, and a quiescent period in winter when the ovaries are inactive and a state of anoestrus prevails. Quinlan, van Rensburg and Steyn (1951) however, have shown that under favourable conditions the mare in South Africa may show periodic oestrus throughout the year, but that many of the oestrous periods, especially during winter (April to July) are sterile. In practice such infertile oestrous periods are frequently encountered during the breeding season as well, and this appears to be one of the most common types of infertility in the mare. In such cases the genitalia are free from infection or anatomical abnormality which may interfere with conception. The mares concerned may have normal oestrous periods during which they may be mated frequently during the breeding season with a normal fertile stallion, and yet they fail to conceive.

The object of the present investigation was to determine the nature of the ovarian activity during such infertile periods and to study methods whereby fertility may be restored in these cases.

### EXPERIMENTAL PROCEDURE.

With the above object in view 24 mares, most of which were presented as reputed shy breeders, were selected. They consisted of ten half Thoroughbreds (Nos. 167, 384, 391, 1010, 1011, 1013, 1015, 1016, 1018 and 1072), eight Percherons (Nos. 435, 776, 1404, 1418, 1595, 1734, 2029 and 2030), and six remounts (Nos. 114, 171, 1096, 1112, 2005 and 2079).

## INFERTILITY IN MARES CAUSED BY OVARIAN DYSFUNCTION.

Observations were made on most of these mares during two breeding seasons (1949-51), but some were in the experiment only for one season. They were stabled at night and ran out in a small enclosure during the day. No grazing was available at any time, and they had to be fed throughout this period. Each received approximately 8 pounds daily of a concentrate mixture consisting of oats, crushed maize and peanut meal, plus 10 to 15 pounds green feed, and lucerne and teff hay *ad lib.*

Clinical examination of the genitalia of the 24 mares revealed no abnormality which could be responsible for infertility. They were teased daily, the onset of oestrus being taken as the first, and termination, as the last day on which the mare accepted the teaser stallion. During oestrus, and at times in dioestrus, rectal palpation of the genitalia was carried out as frequently as was deemed necessary to determine the changes that were taking place in the ovaries.

The data presented in Table 1 cover a total of 52 oestrous periods during which frequent rectal palpation of the ovaries of the 24 mares was performed. The activity shown by the ovaries and the changes that were detected by these examinations are detailed in the last column of the table.

### OESTRUS WITH FOLLICLE FORMATION AND OVULATION.

Since, in all females, maturation of the Graafian follicle ending in ovulation is the main essential for reproduction, any termination of the oestrous cycle other than ovulation results in sterility. Oestrus with follicle formation and ovulation is, therefore, the normal course and termination of every fertile oestrous period.

#### *Results.*

Analysis of the data shows that only 27 of the 52 oestrous periods studied ended in rupture of the follicle. In ten of the 27 cases, however, this was induced by the application of hormone treatment to the mare at the appropriate time. It is, therefore, highly probable that without therapeutical interference more than 50 per cent of these heat periods would have been sterile on account of failure of the follicles to mature and to ovulate.

In the 27 periods which ended in ovulation one mare [No. 1595(2)] was not served during that particular heat period. Two others [Nos. 435(2) and 2005(2)] showed oestrus, and ovulated, while pregnant. In the remaining 24 cases the mares were served on the dates given in Table 1. Conception resulted in 20 out of the 24, thus giving a conception rate of 83·3 per cent for those cases in which oestrus was accompanied by ovulation.

Of the four mares that did not conceive when served during one of these normal oestrous periods, one (No. 2029) was served only once, and that was approximately twelve hours after ovulation.

With five exceptions [Nos. 171(1), 1015(1), 1096, 1418 and 2005] all ovulations took place during oestrous periods of approximately normal duration which did not exceed ten days. It is significant that in four of the five in which the duration of oestrus was unduly prolonged, ovulation was eventually brought about by treatment with hormones. The one exception was the exceedingly long spell of continuous oestrus shown by mare 1418 in which ovulation finally took place spontaneously after an unbroken heat period of 171 days.

*Changes in the Ovaries during Oestrus.*

Although considerable individual variations may occur, the sequence of events which generally takes place in the ovaries during a normal fertile oestrous period is as follows:—

The onset of ovarian activity in one or both gonads is heralded by a change in the consistency of the organ concerned. The previously hard, compressed and fibrous appearance and small size which characterise the anoestrous ovary are rapidly lost, and the gland becomes larger and turgid or spongy. Palpation per rectum on the first day of visible oestrus reveals a small follicle of about 2 cm. diameter projecting slightly above the surface of the ovary. This is firm, almost nodular, and thick-walled, with a high intrafollicular pressure, and is very resistant to efforts to rupture or pitting by digital pressure. Frequently several follicles are present, and they may be all in one ovary or in both.

The follicle which is destined to mature and to rupture may show a consistent increase in size, or may come up suddenly, and it attains a diameter of 4 to 5 cm. or more by the fifth day. On palpation it may then still be firm and resistant. Thinning of the wall of the follicle and an appreciable drop in the intrafollicular pressure, which allows the follicle to become soft and flabby and to pit on pressure, is an indication of impending ovulation, which, in the great majority of cases, will take place within the next 24 hours. The occasional instances in which flabbiness and pitting of the follicle are not followed by ovulation appear to be mainly those in which the follicle has not attained a fair size before softening.

Where several follicles are present they may all show a little development during the first day or two of oestrus, but then become stationary, or may even recede, and only one, or two in the case of twin ovulation, will continue to develop and to rupture eventually.

The experienced examiner has no great difficulty in detecting recent ovulation, since this leaves a well-defined crypt or fossa in the ovary. After about twelve hours this crater become filled with blood and feels soft and yielding. Palpation of the ovary concerned within 24 hours of ovulation invariably provokes pain and causes a mare, which is otherwise very docile and quiet during rectal examination, to show restlessness and resentment as soon as the ovary is touched or even when it is approached with the hand.

Palpation of the ovaries and detection of recent ovulation, with a history of mating up to 72 hours prior to ovulation, warrant an opinion that conception has in all probability resulted.

OESTRUS WITH FOLLICLE FORMATION BUT NO OVULATION.

There is no reliable method excepting rectal palpation of the ovaries whereby one can differentiate between a normal oestrous period and one in which the follicles fail to ovulate. The degree of hyperaemia shown by the vaginal and cervical mucosa, and the extent of relaxation of the cervix and dilatation of the os uteri do provide some indication of oestrogenic secretion and the probability of ovulation taking place. Cases have been encountered, however, in which the changes detected by vaginal examination have justified an opinion that ovulation was impending, but in which the follicle finally regressed instead of rupturing. Neither does the intensity of oestrus provide any evidence. With rare exceptions libido is as pronounced in heat without ovulation as in a normal oestrus.

*Results.*

Graafian follicles developed partially, but failed to mature and to rupture in 23 (44.2 per cent) of the 52 oestrous periods during which manual examination of the ovaries per rectum was carried out while the mare was showing heat.

In 13 of these anovulatory periods service was withheld, but in the remaining ten the mares were mated once or more often on the dates given in Table 1. Naturally not one of these mares conceived to mating during any one of the anovulatory periods.

The duration of oestrus in these abortive periods is also well within normal limits. The data in Table 1 show that three of these periods, namely mares 776 (1), 1072 (3) and 1595, were abnormally long, being 21, 19 and 119 days respectively, but it must be pointed out at the same time that several of the normal heat periods which ended in ovulation and pregnancy were even longer than this. Similarly, irregularities, in that dioestrus was longer or shorter than the average, were shown by some of the anovulatory periods, but these were no greater than many of the aberrations shown by normal ovulating mares.

A good example of irregularities in the sexual cycle is provided by 1072, a maiden mare, born on 8.11.46. She was teased daily for oestrus from 29.10.49 to 13.3.51. During the first season she showed oestrus from 23rd to 30th November 1949. Then in the middle of the breeding season she went into anoestrus after the latter date till 18th April 1950, when a short three-day oestrus was shown, during which there was no evidence of follicular development. This was again followed by anoestrus from 21st April throughout the winter till 6.10.50. A 19-day heat period commenced on this date, and after this the sexual cycle became regular for the remainder of the season. Mating was attempted frequently during these periods but all attempts were unsuccessful on account of impotentia coeundi due to incomplete relaxation of the vulva. Stilboestrol in doses of 15 mgm., 15 mgm. and 25 mgm. on three consecutive days from 18.1.51 was ineffective, and this appeared to be a hopeless case of sterility. On 13.3.51 this mare was turned out in a camp with a stallion and other mares, and when examined two months later, she was found to be six to eight weeks pregnant.

*Discussion.*

The high percentage of oestrous periods in which follicles fail to mature and to ovulate, associated with the absence of any visible symptoms whereby they may be recognised, stamps this type of infertility as one of the most serious breeding problems of equines. Besides preventing conception in the mares concerned, it also constitutes an unnecessary drain on the sire on account of the frequent infertile services which such mares are given. The only method whereby futile matings of this type can be avoided would be by rectal examination by an experienced clinician in order to determine when ovulation is pending, and, where possible, again subsequently to ascertain whether the follicle has actually ruptured.

Failure to ovulate is an abnormality which is not confined to certain mares only, but it may occur in any normal breeding mare at any time under unfavourable environmental, climatic or nutritional conditions. It may be shown during a number of oestrous periods, for instance as in mares 1010 and 1112, in the same breeding season and may then be followed by a normal heat terminating in ovulation and pregnancy. In other cases again anovulation may characterise every oestrous period throughout the breeding season, like mare 776 in 1949-50, or even in two consecutive seasons, such as 1072 in 1949-50 and 1950-51 seasons, but with the onset of more favourable conditions ovulation and pregnancy will result.

A significant fact revealed by the data in the two tables is that of the eleven mares which were responsible for the anovulatory periods, one (1595) was not mated at any time, but the remaining ten all conceived eventually.

#### CYSTIC OVARIES.

Cystic degeneration of the Graafian follicle in the mare is not characterised by a very definite syndrome as in the cow, and does not appear to have received the same intensive study. The available literature and the observations made in this investigation suggest that there is, as yet, great confusion as to the real nature of ovarian cysts in mares, and that normal follicles, which, instead of maturing and rupturing, persist for some time, are frequently mistaken for cysts.

#### *Review of Literature.*

Williams (1943) states that the ovaries of fertile mares present gross variations in structure which do not at present admit of authentic explanation. He describes the ovaries of a group of highly fertile mares as having a large number of cysts up to 1.5 cm. in diameter, but points out that these cysts were in no sense pathological.

Day (1939) divides cystic ovaries in mares into two groups according to the oestrous cycle: mares which do not come into oestrus, and mares continually in oestrus or having long irregular oestrous periods. He points out that cysts cannot be distinguished from follicles by rectal palpation unless the exact time of the oestrous cycle at which the examination is made is known. In several mares with cystic ovaries followed by him through winter, about half went into anoestrus and the cysts disappeared, whereas in the others they persisted.

Burkhardt (1948) points out that many mares push up an outsize follicle in autumn and that these are frequently mistaken for cysts by practitioners. These follicles do not ovulate but persist, and when anoestrus develops they regress and do not recur the following spring. He emphasises that true cystic ovaries are much more difficult to recognise in autumn and winter, and that these mares generally enter a prolonged oestrus in mid-February or early March, and continue to show heat periods of varying durations until the end of the breeding season. The ovaries are enlarged and feel like a bunch of unripe grapes and their surface is studded with a mass of small cysts. According to him no form of treatment at present offers any hope of success for these cases of true cystic ovaries.

Caslick (1937) states that the barren mare with the long oestrous period has been condemned as a mare with cystic degeneration of the ovaries, yet as a foaling mare she becomes a mare with the finest sexual rhythm of any group of mares. Further, the mare with the long dioestrous periods has also been condemned as a mare with cystic degeneration of the ovary.

According to Teunissen (1945) large and small cysts are frequent, even in pregnant mares, and especially in aged animals. He also points out that it is often difficult to differentiate clinically between a normal follicle and a cyst, and that many of the cysts disappear spontaneously.

#### *Results.*

Grape-like follicles or cysts similar to those described by Burkhardt were observed in mare 776 during the 1949-50 season in which her cycles were very irregular. Follicles kept on appearing and regressing in both ovaries. In the beginning of December 1949 she had a large number of follicles in the left ovary

which gave it a grape-like appearance, but these all subsided during the next six weeks, and by the end of January 1950 no follicles or cysts were palpable in any of the ovaries. Despite many services she never conceived that season, and after a five-day heat period in which no follicles at all were palpable, she went into anoestrus on 21.3.50. This continued until 27.12.50 when in a four-day period a follicle in the right ovary ruptured after 10 mgm. Stilboestrol was given, and pregnancy followed two services.

The very marked irregularities in the length of the oestrous and dioestrous periods shown by mares that are otherwise perfectly normal, as reported by Quinlan, van Rensburg and Steyn (1951) and further revealed in the present investigation, emphasise the complete unreliability of variations in the oestrous cycle as a means of diagnosing cystic ovaries without more detailed examination of these organs. For instance, mare 1418 showed continuous oestrus for 171 days during which period there was a succession of follicles coming up and receding without ovulating until eventually one of these matured and ruptured spontaneously a day before the cessation of oestrus. The mare conceived to two services given four and two days before ovulation at the end of this long heat period.

Notwithstanding the very many aberrations in the sexual cycle which were shown at some time or another by most of the mares in this investigation and the frequent examinations that were made during such abnormal periods, not one case which could with any degree of confidence be called a cystic ovary was encountered.

#### *Discussion.*

In the bovine cystic ovaries are characterised by a very definite syndrome (irregularity of the sexual cycle, nymphomania, anaphrodisia and tendency towards masculinity). There is no account available of this sequence being followed in the mare. Moreover, once the follicle in the cow has become truly cystic with degeneration of the ovum, followed by disintegration of the granulosa and the theca interna and externa, there is no possibility of spontaneous recovery, and fertility can be restored, in some cases only, by manual rupture of the follicle and administration of chorionic gonadotrophin. On the other hand, the great majority of mares showing irregular heat periods accompanied by excessive follicular development without ovulation, which it would appear is frequently mistaken for cysts, recover their fertility without treatment as soon as they are placed in a more congenial environment.

In cows there is now a definite tendency originated by Lesbouyries (1944) and subsequently supported by Garm (1949) to regard cystic ovarian degeneration only as a symptom of two different multiglandular syndromes, namely nymphomania and adrenal virilism, in both of which the sexual disturbances are the most important symptoms. In nymphomania the disturbances in the sexual cycle are associated with follicular persistency, the formation of follicular cysts and the absence of a corpus luteum. On the other hand, the masculine sexual behaviour which characterises "buller" cows in adrenal virilism is attributed to a hyperproduction of androgens in the adrenal cortex. This appears to be identical with adrenal virilism in women, which Broster and Vines (1933) treat successfully and get complete reversion to femininity with restoration of fecundity in many cases by means of unilateral adrenalectomy. Garm (1949) and De Lange (1950) have found that in cows which develop masculine characters as the result of cystic ovaries, the adrenal cortex is uniformly enlarged, and Garm has shown that this results in a hyperproduction of androgens. The adrenal cortical hypertrophy in

these cases is but an end result of ovarian cysts, since it is produced by a hypersecretion of the corticotrophic hormone by the anterior pituitary and by the excessive oestrogen derived from the cystic ovary.

There is no record of detailed examination of the ovaries and adrenals in cases of cystic ovaries in the mare, and although nymphomania is frequently encountered no condition which may be regarded as corresponding with adrenal virilism in the cow has, as yet, been described in equines. All the evidence, therefore, suggests that in the great majority of cases of pseudo-cystic ovaries in the mare there are no definite degenerative changes in the follicle, which are characteristic of ovarian cysts, and that in most cases the condition is rather one of a transient persistence of one or more follicles which ultimately regress and become atretic or ovulate when conditions become more favourable.

#### PROLONGED OESTRUS.

The average duration of oestrus in the mare is approximately six days, but all workers who have studied this aspect draw attention to the great variations that may occur. Thus Crowhurst and Caslick (1946) found a range of one to 130 days. For mares maintained under similar South African conditions as those in the present experiment Quinlan, Van Rensburg and Steyn (1951) determined an average duration of 6.93 days with a range of one to 55 days.

#### *Results.*

The 52 periods involved in this investigation were of an average length of 12.9 days. This abnormally high mean is due to the two exceptionally long periods of 119 and 171 days. If these are excluded the average for the remainder is 7.5 days.

The duration of oestrus exceeded ten days in the following eight of the 52 periods:—

Mare No.	Duration of Oestrus.	Termination of Oestrus.
171	14 days	Ovulated after treatment; mated and conceived.
1015	16 days	Ovulated after treatment; mated and conceived.
1072	19 days	No ovulation; follicles regressed.
776	21 days	No ovulation; follicles regressed.
1096	23 days	Ovulated after treatment; mated and conceived.
2005	29 days	Ovulated after treatment; mated and conceived.
1595	119 days	No ovulation; follicles regressed.
1418	171 days	Ovulated spontaneously; mated and conceived.

#### *Discussion.*

It is generally believed that, with the continuation of oestrus beyond the average duration, the possibility of conception is decreased. The presumption is confirmed by the above results, notwithstanding the fact that five of the eight long periods ended in pregnancy, as it must be pointed out that in four of the five ovulation was eventually induced by hormone treatment. It is noteworthy that the only period of the eight which ended in spontaneous ovulation and pregnancy is the one (1418) which was the longest (171 days).

## INFERTILITY IN MARES CAUSED BY OVARIAN DYSFUNCTION.

The immediate cause of prolonged oestrus is failure of one or more follicles, which are responsible for heat, to rupture, obviously on account of defective luteinisation. Even under normal conditions, the gonadotrophic secretions of the anterior pituitary in equines are heavily loaded in favour of the follicle stimulating hormone, and unfavourable conditions may still further depress the luteinising factor, with the result that there is an insufficiency to bring about maturation and rupture of the follicles.

The sequel to this loss of equilibrium does not appear to be the same in the mare as in the cow. In the latter, the usual course is for the primary follicle or follicles to continue growing and, finally, to lead to the development of true sterility. In mares, on the other hand, the abnormally high preponderance of F.S.H. over L.H. is not usually characterised by continued growth and eventual cystic formation of one or a few follicles. Rather successive waves of follicles appear in one or in both ovaries, show a little development, but before reaching maturity, regress again, only to be followed by the appearance of more follicles which maintain the clinical symptoms of oestrus.

This process continues for a variable period and may terminate in maturity and rupture of one or more follicles, as happened at the end of the long oestrus shown by mare 1418, or the process may stop when the ovaries become inactive. After a period of rest, and particularly with the onset of more favourable conditions, the normal sexual cycle may be resumed.

Instead of waves of follicles following each other, only one or two may be pushed up by the ovaries and develop to a stage when they are nearly on the point of rupturing, but then become static for some days. Treatment at this stage may induce ovulation, otherwise they recede after a while.

A significant feature of these prolonged heat periods is that the two longest, namely those of 119 and 171 days, occurred in two young maiden mares. This and the deviations from the normal shown by mare 1072 already described, suggest that the cycle is particularly erratic in maidens, and that it apparently takes some time for proper hormonal equilibrium to be established after the filly has reached puberty. This would depend to a very large extent on the management and feeding of the fillies. Burkhardt (1948) states that immaturity is probably responsible for more cases of barrenness in maiden mares than generally recognised, and while some mares become pregnant as yearlings, others may not produce mature follicles until they are five years old or even older.

### OESTRUS WITHOUT FOLLICLE DEVELOPMENT.

The normal manifestations of oestrus are due to the oestrogen secreted by the developing follicle. There is evidence however of symptoms of oestrus being shown without follicular formation.

#### *Review of the Literature.*

Maheffey (1950) reports having encountered many mares with intense sex desire but showing no trace of follicle development or ovarian activity of any kind. Hancock (1948) quotes Hignett as stating that nymphomania may be encountered in mares showing no obvious ovarian dysfunction and that ovariectomy frequently fails to terminate this type of oestrous behaviour. Hancock further mentions the possibility that oestrogen may be produced at some site other than the ovary. According to Robson (1947) oestrogen can be extracted not only from the ovary and the placenta but also from many other tissues, including testes,



adrenals and the pituitary. He also points out that, in humans, oestrogens are secreted before puberty and that they may play a part in pre-pubertal development of the secondary sex organs. The revelation by Bennetts (1946) that certain strains of subterranean clover contain large quantities of oestrogenic factors which produce marked symptoms in sheep suggests the possibility of small amounts of oestrogens being derived from other plants as well.

### *Results.*

In two of the 52 heat periods in which the ovaries were palpated it was not possible to detect any sign of follicular growth in any one of the two ovaries during the whole period the mares were in oestrus.

In an apparently normal seven-day period from 18th to 24th January 1950 mare 776, which had developed follicles that failed to rupture in both the immediately preceding periods, displayed all the usual clinical symptoms of oestrus, but rectal palpation of the ovaries failed to reveal any sign of follicular growth. The right ovary was firm and static throughout while the left was somewhat enlarged and turgid with a smooth surface. The vaginal and cervical mucosa was moist and slightly hyperaemic, but there was no dilatation of the os uteri. She accepted service on the second, fourth and last day of oestrus but did not conceive. In her two subsequent periods, follicles developed but did not mature or ovulate. Therefore, in the five periods in which her ovaries were examined during the 1949-50 season she did not ovulate once, despite the fact that chorionic gonadotrophin was given intravenously during one of these periods. She went into anoestrus on 21 March, 1950, for the winter. In the following season during a four-day heat period starting on 27 December, 1950, and in response to 10 mgm. Stilboestrol she ovulated within 24 hours, was served and conceived.

The other mare to show oestrus in the absence of follicles was 1072. As is apparent from the data in Table 1 she had very irregular cycles during the 1949-50 season in which she only came into heat twice, namely from 23rd to 30th November, 1949, and for three days from 18th April, 1950. The first period was characterised by the presence of follicles in both ovaries, but in the second the ovaries were small, smooth and firm with no detectable evidence of activity of any kind. The vaginal and cervical mucosa was blanched and dry and the os uteri firmly closed. Notwithstanding these negative signs, she showed all the psychical symptoms of oestrus and stood to service. From 20 April, 1950, she was in anoestrus through the winter till 6 October, 1950, and from the later date her cycles for that season were very regular. She conceived before the end of the season (Table 2).

### *Discussion.*

No satisfactory explanation can be given for this display of true oestrus while the ovaries appeared to be in a complete state of anoestrus and not secreting any oestrogen. In the case of 776 there was still some sign of activity in that the left ovary was slightly enlarged and softish. This points to the possibility of small follicles developing in the substance of the ovary without being palpable on its surface. In mare 1072 there was, however, no indication whatever of follicle formation or secretion of oestrogen by the ovaries.

The possibility of an extra ovarian source of oestrogen may account for the oestrus shown by mare 776. In the case of 1072, however, there was no sign, excepting libido, of any oestrogenic activity. In explaining this case, therefore cognisance must be taken of the possibility of psychological factors being

## INFERTILITY IN MARES CAUSED BY OVARIAN DYSFUNCTION.

responsible for the libido, and reference must be made to the possible influence of neutral control which may play a far more important rôle, and exert a greater influence on the sexual cycle in equines, than is generally believed.

### FOLLICLE DEVELOPMENT WITHOUT OESTRUS.

The development, maturation and ovulation of Graafian follicles without any clinical signs of oestrus or evidence of sexual desire, commonly known as silent heat, is a fairly common occurrence and a frequent cause of infertility in bovines. Available literature, however, contains very little information on this type of deviation from the normal oestrous cycle of mares.

Day (1939) states that it has been found in a few mares that ovulation may take place without oestrus, and oestrus without ovulation. Hancock (1948) reports that failure to show normal cyclic oestrous behaviour was recognised in three mares.

#### *Results.*

Since the main object of the present investigation was to determine the activity within the ovaries during actual oestrus, no routine rectal palpation was performed during dioestrus. In a few cases, however, in which marked irregularities in the cycles were shown such examinations were made, and in this manner the development of fair-sized follicles unaccompanied by visible oestrus was detected in three mares (1011, 1018 and 2004). This did not happen during the usual quiescent period in winter, but during periods of anoestrus that were shown while the breeding season was at its height.

A remarkable similarity was shown in the aberrations in the oestrus cycles of mares 1011 and 1018. Both ran together in the same paddock where they were teased daily from 6th September, 1949 to 13th March, 1951. During the whole of this 18 month period they were in anoestrus except for a slight heat period shown by both on the same three days (18th to 21st January, 1951). Although their ovaries were static for most of the 18 months, both developed follicles at various times during the two breeding seasons, but in every case these receded without rupturing.

The observations have shown that a mare generally does not show oestrus until the follicles have attained a diameter of 2-3 cm. In the majority of cases the follicles in these two mares regressed before they reached that size. In the 1949-50 season, however, each showed follicles of 4 and 4.5 cm, respectively, without any indication of oestrus. The follicles also receded without ovulating.

Mare 2004 was teased daily from 8th January, 1951 to 13th March, 1951 but never showed evidence of oestrus. In the middle of January, a week after the commencement of teasing, a firm, thick-walled follicle which attained a diameter of 5 cm. developed in the left ovary, but regressed again without the mare showing heat at any time.

These findings indicate that silent heat may be a fairly common feature in cases of anoestrus in mares during the breeding season.

The data in Table 2 reveal the significant fact that all three these mares that showed this sluggish ovarian activity conceived within two weeks after they were turned out in a camp with a stallion towards the end of the 1950-51 breeding season.

### RELATIVE ACTIVITY OF THE OVARIES.

In the cow the right ovary is far more active than the left, and most pregnancies occur in the right cornu of the uterus.

A few investigators have attempted to ascertain whether the mare shows a similar functional disparity between the two ovaries.

#### *Review of the Literature.*

According to Amoroso, Hancock and Rowlands (1948) ovulation in the mare occurred more frequently in the left ovary. They observed that 22 out of 36 ovulations were in the left and 14 in the right ovary. Similarly, Hancock (1948) found that in 35 instances of single ovulation 22 occurred in the left ovary. Day (1940) states that ovulation does not occur alternately in the left and then in the right ovary, but the frequency of ovulation in either is approximately equal. In his mares the left ovary ovulated 13 times to 18 times in the right.

#### *Results.*

In the present study 28 ovulations were observed in the 52 oestrous periods. One was a case of twin ovulation. Of the remaining 27, 16 were in the right and eleven in the left ovary.

#### *Discussion.*

These results are more in accordance with those obtained by Day, and consideration of all the observations quoted justifies the view that, contrary to what pertains in the bovine, in which the disproportion is attributed to anatomical differences such as the large rumen on the left side, there appears to be no significant difference between the two ovaries of the mare as regards their functional activity. Our findings also agree with those of Day in that ovulation does not necessarily take place alternately in the two ovaries. One may remain static while the other functions for several periods. In other cases again, both are active and push up follicles at the same time, but in the end only one of these follicles ruptures in either the right or the left ovary.

### OVULATION IN RELATION TO THE END OF OESTRUS.

It is generally accepted that the optimum time for serving a mare is during the 24 hours immediately preceding ovulation, and that ovulation usually occurs during the final 24 hours of oestrus. If, therefore, the breeder can be furnished with a reliable method of determining the two most essential factors, namely, whether oestrus, when present, will terminate in ovulation, and, secondly, *when* this will take place, he may be able to get every mare into foal with only one service. This would result in great economy of the use of sires, and may enable a good stallion to settle two or three times as many mares in a season as at present.

The extreme variations which may occur in the duration of oestrus and the frequency with which follicles fail to ovulate present an almost insurmountable obstacle to the solution of this problem. While the mean duration of oestrus is five to seven days three cases (mares 1096, 2005 and 1418) have already been cited, in which oestrus lasting 23, 29 and 171 days respectively ended in ovulation and conception. Any attempt, therefore, to confine ovulation to a specified period from the beginning of oestrus would be futile.

*Results.*

In most of the cases in which oestrus ended in ovulation in this work it was observed that rupture of the mature follicle was in accordance with the findings of other workers in that it took place during the last 24 to 48 hours of heat. In two, however, oestrus was continued for several days after ovulation. Mare 1016 ovulated and was served successfully on 3rd October, 1949 but continued to show heat for another four days. Mare 1734 took a fertile service on 22nd December, 1949 and ovulated within 24 hours, but strong oestrus persisted till 28 December, 1949. In each of these two cases it was observed that a large follicle was also developing in the other ovary and continued to grow for some days after the primary follicle had ruptured. These secondary follicles were apparently responsible for the secretion of sufficient oestrogen to maintain symptoms of heat for some time after the mature follicles had ovulated.

OPTIMUM TIME FOR SERVICE.

Of great practical importance too is the maximum period between service and ovulation which may be allowed for conception. If it is established that fertile services can be obtained only by mating within 24 hours of ovulation, it will necessitate daily serving throughout oestrus to ensure pregnancy. This will entail an unnecessary drain on the sire.

*Review of Literature.*

In his observations on 1,500 cycles of mares at the United State Army Remount Depot Trum (1950) found that all fertile matings take place before ovulation. Day (1940) states that inseminations within 72 hours of ovulation are equally effective whereas inseminations in the interval of two to four hours after ovulation were not effective.

*Results.*

In the present investigation 24 of the mares that ovulated were mated, with the result that 20 conceived and four failed. Of the 20 pregnant mares two were mated for the last time 72 hours before ovulation, six at 48 hours, and the remaining twelve within the last 24 hours prior to ovulation. Three of the four that did not conceive were served 48 hours before ovulation, while the fourth (No. 2029) had only one service approximately eight to twelve hours after ovulation. These results confirm that, in the absence of other factors which may prevent conception, pregnancy should result from all matings which take place within 72 hours before ovulation, and that post ovulatory services appear to be sterile.

*Discussion.*

Since there is no reliable method of determining beforehand how long oestrus will last, or when ovulation is likely to take place, other than by palpation of the ovaries, this is obviously the only reliable manner in which the best time to mate each individual mare can be ascertained. In the absence of such examination, mating once every three days during heat should suffice.

In this connection cognisance must be taken of the fact that no generalisation is possible as regards the oestrous cycle of the equine and that each mare must be treated as an individual entity. The size and consistence of the maturing

follicle just prior to ovulation have been described, but it must be pointed out that, where possible, another examination should be made a day or two later to ensure that ovulation has in fact taken place.

As previously indicated, exceptional cases do occur in which follicles develop up to a point when ovulation appears to be imminent, but which, instead of rupturing, remain static or regress.

The determination of the best time for service by examination of the vagina and cervix by means of a speculum, besides not being infallible, is considered too dangerous a method for general application, especially by laymen. The speculum may be a very potent carrier of infection into the vagina and uterus. Even the application of scrupulous antiseptic measures with regard to the speculum provides no absolute safeguard, since mere dilatation of the vagina during the ovulation period, when the os uteri is wide open, may result in air laden with bacteria being aspirated into the uterus to prevent conception.

#### OESTRUS AND OVULATION DURING PREGNANCY.

The occurrence of oestrus during pregnancy is not an uncommon feature in the mare, but not many cases of ovulation during such periods have been recorded.

##### *Review of the Literature.*

Day (1940) reports ovulation in a mare on the 23rd day of pregnancy, but the mare did not show any signs of oestrus.

Amoroso, Hancock and Rowlands (1948) point out that the corpus luteum of pregnancy is short-lived in the mare, and that it regresses at about the end of the first month. This is accompanied by great ovarian activity during the second and third months, which is characterised chiefly by follicular development. It is highly probable that during this stage a succession of follicles ovulate in order to provide fresh corpora lutea to keep up the gestational requirements of progesterone after regression of the primary corpus luteum of pregnancy.

##### *Results.*

Two cases of oestrus with ovulation during pregnancy were observed in the present study. Mare 345 showed a normal oestrus from 7th to 14th December 1950, in which a follicle in the left ovary ruptured on the last day. She conceived to a service given on the 12th. Six weeks later, however, she again showed strong heat for three days. During this period a follicle developed rapidly in the left ovary to a diameter of about 6 cm. on the second day of oestrus and ruptured on the third day.

Mare 2005 was served successfully on the 28th October 1950 during a five-day period in which rupture of a 7 cm. follicle in the right ovary was induced with stilboestrol. Eight weeks later she was again on heat. Rectal examination on the first day revealed an eight-week old foetus in the right cornu and a follicle of 6 cm. in the corresponding ovary. This follicle ruptured three days later.

Further evidence on ovulation and the formation of corpora lutea during early pregnancy was provided by post mortem examination of three mares, namely 1488, 1479 and 1482. The data in Table 2 show that these three old mares, which were in poor condition, all conceived towards the end of the 1950-51 breeding season, when they were running out in a camp with the stallion. When examined

for pregnancy on 18th May 1951 they were found to be eight weeks, six weeks, and five weeks pregnant respectively. In each case the clinical diagnosis of pregnancy was confirmed by the Ascheim-Zondek test.

Mare 1488 was killed on 1st June, 1951. Examination of her genitalia showed a ten-week old foetus in the left uterine cornu and two corpora lutea of 2 and 3.5 cm. diameter respectively in the corresponding ovary. The older and smaller of the two was obviously the primary corpus luteum of pregnancy which was now regressing. The other large one was judged to be one to two weeks old and was presumed to be taking over the function of the former which was receding.

Mare 1479 was slaughtered on 2nd July, 1951. There was no sign of a foetus in the uterus, and she had apparently aborted while running out in a camp in the period between 18th May and 30th June. Her left ovary had a corpus luteum, 2 cm. in diameter and dark brown in colour, evidently the primary corpus luteum of pregnancy.

Mare 1482 aborted an eight-week old foetus on 4th June, 1951. She too was slaughtered on 2nd July. Her right ovary had a dark brown corpus luteum  $2\frac{1}{2}$  cm. in diameter and an adjacent firm thick-walled follicle of the same size.

#### *Discussion.*

A significant fact revealed by these three mares is that No. 1488, in which pregnancy was running a normal course, had ovulated during pregnancy and had formed an active corpus luteum to replace the primary body which was receding. The other two mares which had aborted both had only the primary corpus luteum of pregnancy, and in both this was obviously regressing. Mare 1482 had developed another follicle which, however, had failed to mature and rupture, thus providing a second corpus luteum. It would appear that the immediate cause of the abortions in these two mares was failure on the part of their ovaries to produce fresh corpora lutea after conception, and in this manner to keep up the supply of progesterone necessary to maintain gestation. This failure of the ovaries to form fresh corpora lutea soon after conception may be a more frequent cause of death and resorption of the foetus, or early abortion, in mares than is generally recognised.

#### HORMONE TREATMENT.

With endocrine disturbance constituting the fundamental factor in the aetiology of infertility caused by ovarian dysfunction, the logical presumption is that it should be possible to overcome this form of sterility by administering the appropriate hormones. This is not borne out by the results in practice. After extensive investigations in both bovines and equines, the consensus of opinion appears to be that hormones are only effective when used judiciously in selected cases, at the right time and in the correct dosage.

#### *Results.*

Treatment with oestrogen in the form of stilboestrol dipropionate in oil, and with two brands of chorionic gonadotrophin, namely prolan and luthormone, was applied in 17 cases with the object of producing maturation and rupture of the Graafian follicles in mares showing heat, but in which there was reason to suspect that the oestrus would not terminate in ovulation.

Details of the animals treated are provided in Table 1. Ovulation was induced in the following ten mares out of the 17 treated, namely, 167, 171, 384 (3), 391 (1), 391 (3), 776 (6), 1015, 1016, 1096 and 2005. All ten mares were mated at the time and only one failed to conceive.

#### *Discussion.*

In an evaluation of these results due allowance must be made for the possibility that some of the mares might have ovulated without treatment. On the other hand it must be pointed out that these cases were selected for treatment on account of some abnormality, either in the oestrous cycle or in the breeding history, which gave rise to a suspicion that the heat period might not end in ovulation. For instance, in some, follicles developed up to a stage and then became static, and experience with the palpation of ovaries during oestrus has shown that if the follicle does not continue to show steady growth until it becomes soft with a drop in intrafollicular pressure preparatory to ovulating, the probability of it regressing instead of rupturing is very great. In other cases again the mares were reputed shy breeders or barren, or had shown deviations from the normal in preceding oestrous periods. Further, as has already been pointed out, four were treated on account of undue prolongation of the heat period concerned.

Of the seven failures, three (1011, 1018 and 1072) each received three injections of stilboestrol on three successive days, the total dosage for each amounting to 45 mgm., 45 mgm. and 55 mgm. respectively. The effect of these large doses of oestrogen was exactly the opposite to that expected. Instead of an intensification of the symptoms of oestrus causing it to end in ovulation, the administration of the big doses was followed by regression of the follicles and disappearance of oestrus in every case. This is in accordance with Burkhardt's contention (1947) that the dosage of oestrogen should be carefully regulated, varying from 5 to 15 mgm. for maiden and barren mares, and that bigger doses may depress the pituitary and cause follicular atresia.

Smaller doses of stilboestrol (10 and 15 mgm. respectively), on the other hand, failed in two cases, namely mare 1418 and 1112, both maidens with very irregular cycles. In 1418 10 mgm. was given on the 115th day of the exceedingly long 171-day period, and it should be noted that this ended eight weeks later in ovulation and conception. Although mare 1112 also showed no immediate response, she nevertheless ovulated and conceived during the heat period immediately following that in which stilboestrol was injected. It is not claimed that the good results which ultimately followed treatment in these two cases were produced by the stilboestrol, but in view of the many puzzling features which frequently accompany the use of this oestrogen, the possibility of its action in jolting the anterior pituitary and thus exerting an indirect or delayed beneficial effect cannot be overlooked.

The remaining two failures were both treated with prolan. Here on the other hand there is the possibility that the dose was too small. The good results obtained with large doses of luthormone suggest that, contrary to what applies to oestrogen, a fairly large dose of chorionic gonadotrophin (1,000-2,000 i.u.) should be given to produce ovulation.

It is concluded from these results that beneficial results can be expected from the use of either oestrogen or chorionic gonadotrophin for cases in which the development of the follicle during oestrus appears to be sluggish and not likely to end in ovulation. In resorting to this form of treatment the sexual and breeding history of the mare should be considered, and examination of the ovaries per rectum to determine the degree of development shown by the follicle should in all cases precede treatment.

EFFECT ON FERTILITY OF RUNNING MARES WITH THE STALLION.

According to Day (1939) the fertility rate in different groups of horses in Britain is: Heavy horses, 59 per cent; light horses, 52 per cent; thoroughbreds at stud, 68 per cent; and ponies running wild, 95 per cent. Mahaffey (1950) quotes Anderson as giving the percentage for Kentucky as 50-60. For Holland Teunissen (1945) regards a rate of not higher than 50 per cent as normal for farm animals, though it may be higher in studs.

In order to determine whether running mares with the stallion under natural conditions would result in a higher conception rate than teasing and hand-serving under stabled conditions, it was decided during the second half of the 1950-51 breeding season to turn out all the mares that had not conceived with two stallions in two different camps.

Twenty-four mares were selected including eight that were used in the present investigation. Only one (No. 1734) of the latter eight was a normal foaling mare. The breeding history and the irregularities in the sexual cycle (see Table 1) of the other seven were so bad that most were regarded as hopelessly sterile. Of the remaining 16 mares 14 were either barren or shy breeders, only two being considered normal breeders (Table 2.).

The mares were divided into two lots of twelve each. Group A were placed with Percheron stallion 2039 in a camp of about 200 acres, and Group B with thoroughbred stallion 2010 in another camp of approximately 300 acres. Both stallions had previously been used for hand-serving the same mares while they were stabled. No supplementary feeding of any type was given, and the grass in the two camps was poor as both had carried cattle during the summer. The animals were thus left to run under completely natural conditions for periods varying from four to twelve weeks.

The numbers of the mares, their age, breeding history, period for which they were out with the stallion and the result of the subsequent pregnancy examination are detailed in Table 2.

*Results.*

On the dates specified the mares were removed from the stallions and brought back to the stables. Examination per rectum for pregnancy was carried out on all 24 on 18th May, 1951. In all cases of doubt the clinical findings were corroborated by the Asheim-Zondek biological test, and where necessary a re-examination was made three weeks later. The results show that 21 out of the 24 (87.5 per cent) mares conceived during the period they were running free day and night with the sire.

*Discussion.*

The significance of the very high conception rate of 87.5 per cent obtained in this experiment is enhanced by the following facts:—

- (1) With three exceptions all the mares previously had proved difficult to settle.
- (2) They were turned out with the stallion towards the end of the breeding season when, according to Quinlan, Van Rensburg and Steyn (1951), the fertility rate is low.
- (3) The grazing was comparatively poor, and most of the mares lost condition. Nutritional factors, therefore, cannot be regarded as playing a rôle.



Another important fact revealed by the data in Table 2 is that, with only one or two exceptions, all the pregnant mares appear to have conceived within the first two or three weeks of being turned out. The hormonal imbalance which was responsible for the different types of ovarian dysfunction that prevented conception earlier in the season, therefore, appears to have adjusted itself in a remarkably short time after the animals were turned out and placed in more congenial surroundings.

The inescapable conclusion to be derived from these results is that permitting equines to breed under completely natural conditions is still our most effective method of dealing with all types of infertility caused by functional aberrations in the ovaries. It is thus suggested that the high fertility rate recorded by Day for ponies in Britain is not due to any inherent characteristic of the breed as such, but rather to the more favourable environmental conditions under which they breed. This high fertility rate would most probably show a sharp decline if the ponies were more domesticated and pampered. Conversely, the conception rate in the Thoroughbreds would be greatly increased by allowing them to reproduce under more natural conditions.

Equines are not subject to such a large number of pathological conditions and infective venereal diseases, that produce sterility, as bovines. The most frequent causes of infertility in horses are physiological factors, and in all efforts to determine the various conditions which may produce abnormal physiological functioning of the equine ovary the psychological aspect must receive proper consideration. The equine without doubt shows a far higher psychological development than the bovine, and in no other aspect is this superior mental state of the horse more prominent than in breeding. During the mating season there is far more courtship and "love play" between the two sexes in equines than in bovines, particularly when allowed to roam together in the natural state. It is evident from these results that contravention of the rules of nature and interference with the natural instincts by introducing too much artificiality into our methods of breeding cannot be carried out in equines with the same impunity as in cattle.

#### SUMMARY.

(1) The ovaries of 24 mares were examined rectally during a total of 52 oestrous periods in the 1949-51 breeding seasons. Only 27 of the 52 periods ended in ovulation. In 24 of the 27 normal periods the mares were mated and conception resulted in 20 (83.3 per cent).

(2) Follicles developed but then regressed without ovulating in 23 (44.2 per cent). Ten mares mated during anovulatory periods all failed to conceive.

(3) It is suggested that many cases of pseudo-ovarian cysts in the mare are not real cysts but follicles which persist for a time and then regress, or ovulate when conditions become favourable.

(4) Two cases in which oestrus was shown continuously for 119 and 171 days respectively are recorded. In the latter the prolonged period of heat terminated in ovulation and conception.

(5) Another two cases were noted in which oestrus occurred without follicle development. Attention is drawn to the possibility of extra ovarian sources of oestrogen, and of neural control over the sexual cycle.

(6) Marked follicular development without symptoms of oestrus was observed in three cases.

## INFERTILITY IN MARES CAUSED BY OVARIAN DYSFUNCTION.

(7) No significant difference in the relative activity of the right and left ovaries was shown.

(8) In the great majority of cases ovulation, when present, occurred within the last 24-48 hours of oestrus, but in two mares heat persisted for four and five days respectively after ovulation. This is attributed to the presence of other follicles.

(9) Pregnancy resulted when mares were served up to 72 hours before ovulation. One mare which was mated only once, at 8-12 hours after ovulation, did not conceive. Palpation of the ovaries per rectum is considered the only satisfactory method of determining the most suitable time for service.

(10) Two cases of oestrus with ovulation during pregnancy were noted. At post mortem one mare with a ten week old foetus had two corpora lutea, the one regressing and the other coming up to take over its function. Two other mares, that had aborted during the second month of pregnancy, each only had the primary corpus luteum of pregnancy. Failure of the ovaries in these two cases to form fresh corpora lutea in order to maintain the necessary level of progesterone is suspected to be the cause of the abortions.

(11) Treatment with stilboestrol dipropionate and chorionic gonadotrophin was applied to 17 mares which showed irregularities in the sexual cycle. This was followed by ovulation in ten, and nine of these conceived. Hormone treatment should only be applied after proper rectal examination of the ovaries, in certain selected cases, and dosage and time of administration should be carefully calculated.

(12) Late in the second half of the 1950-51 season 24 mares, all of which but three were shy breeders or barren, were turned out to run free with two stallions in two camps. Within two months 21 (87.5 per cent) had conceived. This high conception rate is attributed to psychological factors.

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INFERTILITY IN MARES CAUSED BY OVARIAN DYSFUNCTION.

TABLE 1.

No. and Age of Mare.	Breeding History.	Oestrous Periods in which Ovaries were Examined.		Duration of Oestrus in Days.	Termination of Oestrus.	Stage of Oestrus in which Mares were Mated.	Result of Mating.	Changes noted in Ovaries during Oestrous Periods.
		From.	To.					
114 17 years	Normal foaling mare	(1) 23/11/49	27/11/49	5	Ovulation....	2nd and 4th days	No conception	Small follicle, 3 cm., in R.O., soft and flabby on 4th day and ruptured within 24 hours. Follicle of 2 cm. on 1st day increased, and on 5th day was 5 cm., soft, pitting, thin-walled; ovulated same day.
		(2) 13/12/49	17/12/49	5	Ovulation....	3rd and 5th days	Pregnant.....	
167 11 years	Had 2 foals in 8 years; showed normal oestrous cycles in past 2 seasons	(1) 7/10/49	15/10/49	9	Ovulation....	1st, 6th and 9th days	Pregnant.....	Follicle developed in each ovary; that in L.O. ruptured on 9th day, while the other receded.
		(2) 29/11/50	6/12/50	8	Ovulation....	4th, 6th and 7th days	Pregnant.....	Large firm follicle, 7 cm., in L.O. on 2nd day; remained static for 5 days; 15 mgm. stilboestrol on 7th day caused drop in intrafollicular pressure in 24 hours and ovulation within the next 24 hours.
171 11 years	Normal foaling mare	(1) 28/9/49	11/10/49	14	Ovulation....	3rd and 11th days	Pregnant.....	Several small follicles present in both ovaries on 1st day. Showed no change in next 2 days; prolan 500 i.u. given 3rd day. In next 5 days a large follicle developed in R.O. while all others regressed; luthorone 500 i.u. given on 11th day was followed by ovulation within 48 hours.
		(2) 9/12/50	15/12/50	7	Ovulation....	3rd, 5th and 6th days	Pregnant.....	A follicle of 9 cm. had developed in L.O. by 5th day; 10 mgm. stilboestrol that day caused it to rupture in the next 24 hours.

TABLE 1 (continued).

No. and Age of Mare.	Breeding History.	Oestrous Periods in which Ovaries were Examined.		Duration of Oestrus in Days.	Termination of Oestrus.	Stage of Oestrus in which Mares were Mated.	Result of Mating.	Changes noted in Ovaries during Oestrous Periods.
		From.	To.					
384 15 years	Sent in as a shy breeder; oestrous cycles very irregular with long dioestrous periods. Had 3 foals in 9 years	(1) 28/9/49	4/10/49	7	No ovulation	2nd, 3rd, 4th and 6th days	No conception	1st day R.O. had firm follicle, 3 cm., and L.O. several small ones; they showed no great change for 3 days; prolan 500 i.u. on 3rd day ineffective, nor were 750 i.u. on 6th day; after this all follicles regressed. A 3 cm. firm follicle in L.O. on 1st day failed to develop further and receded.
		(2) 27/11/49	29/11/49	3	No ovulation	3rd day....	No conception	Follicle in R.O. grew up to 5 cm. in first 6 days; remained firm; luthormone 1,500 i.u. on 7th day caused softening and pitting in 24 hours. Next day it had ruptured leaving fossa, and pain shown on manipulation of ovary.
		(3) 14/12/49	22/12/49	9	Ovulation....	4th, 7th and 9th days	Pregnant.....	
391 12 years	A shy breeder with 3 foals in 9 years	(1) 12/10/49	15/10/49	4	Ovulation....	1st day....	No conception	Follicles of 2-3 cm. present in both ovaries on first day; stilboestrol 15 mgm. caused one in R.O. to burst within 24 hours, but oestrus continued another 48 hours.
		(2) 29/10/49	2/11/49	5	No ovulation	3rd and 4th days	No conception	Several follicles up to 5 cm. in both ovaries, but all regressed.
		(3) 15/12/50	22/12/50	8	Ovulation....	6th and 8th days	Pregnant.....	Follicle in R.O. grew to 6 cm. on 7th day when luthormone 1,500 i.u. was given. Ovulation followed in 24 hours.

INFERTILITY IN MARES CAUSED BY OVARIAN DYSFUNCTION.

TABLE 1 (continued).

No. and Age of Mare.	Breeding History.	Oestrous Periods in which Ovaries were Examined.		Duration of Oestrus in Days.	Termination of Oestrus.	Stage of Oestrus in which Mares were Mated.	Result of Mating.	Changes noted in Ovaries during Oestrous Periods.
		From.	To.					
435 10 years	Poor breeder with irregular cycles and long periods of anoestrus	(1)	7/12/49	14/12/49	Ovulation....	6th day....	Pregnant.....	A 2.5 cm. firm thick-walled follicle in L.O. on 1st day was 6 cm. soft and pitting on the 6th day and ovulated within the next 48 hours Follicle 4 cm. and firm on 1st day was 6 cm. and soft 2 days later and ovulated within the next 24 hours. Note mare was already pregnant to previous service.
		(2)	23/1/50	26/1/50	Ovulation....	2nd day...	Accepted while pregnant	
776 14 years	Shy and barren; had not foaled for 6 years. Very irregular oestrous cycles	(1)	11/11/49	3/12/49	No ovulation	7 times....	No conception	Small follicles present in both ovaries on 2nd day. One in L.O. grew to 4 cm. and was soft on 8th day but then receded. Another of 3 cm. in R.O. was soft and pitting on 19th day but also receded. A bunch of follicles of 2-3 cm. in L.O. on 20th day, but none developed further. Oestrus returned after 4 days due to follicle growing in L.O. This reached 4 cm. on 6th day, was then soft and fluctuating, but regressed. Prolan 300 i.u. on 5th and 450 i.u. on 6th days failed to cause ovulation. No follicle could be detected in any of the ovaries, but L.O. was large and turgid. Follicle in R.O. grew up to 6 cm. on 3rd day; then suddenly receded. Follicles up to 4 cm. in both ovaries, but regressed after 3rd day. Follicles of 8 and 7 cm. in R.O. and L.O. respectively. That in R.O. was soft and pitting on 3rd day and ruptured within 24 hours after given 10 mgm. stilboestrol. The left subsided.
		(2)	7/12/49	15/12/49	No ovulation	6th day....	No conception	
		(3)	18/1/50	24/1/50	No ovulation (no follicle formation)	2nd, 4th and 7th days	No conception	
		(4)	8/2/50	11/2/50	No ovulation	4th day....	No conception	
		(5)	17/3/50	21/3/50	No ovulation	Not served	—	
		(6)	27/12/50	30/12/50	Ovulation...	3rd and 4th days	Pregnant.....	

TABLE 1 (continued).

No. and Age of Mare.	Breeding History.	Oestrus Periods in which Ovaries were Examined.		Duration of Oestrus in Days.	Termination of Oestrus.	Stage of Oestrus in which Mares were Mated.	Result of Mating.	Changes noted in Ovaries during Oestrus Periods.
		From.	To.					
1010 13 years	Shy breeder. Had 1 foal in 5 years. Irregular oestrus	(1) 24/10/49	27/10/49	4	No ovulation	Not served	—	A firm follicle in L.O. attained $3\frac{1}{2}$ cm. and then receded.
		(2) 5/12/49	10/12/49	6	No ovulation	1st, 3rd and 6th days	No conception	A firm thick-walled follicle in R.O. got soft and flabby on 3rd day but regressed.
		(3) 14/1/50	16/1/50	3	Ovulation....	3rd day....	Pregnant.....	Follicle developed rapidly in R.O., was 6 cm. on 3rd day and ovulated.
1011 12 years	Sent in in 1946 as a shy breeder. Has not foaled since arrival	18/1/51	20/1/51	3	No ovulation	Not served	—	Mare was teased daily for 18 months (6/9/49 to 13/3/51). Was in anoestrus all the time except for slight heat from 18 to 20/1/51. Ovaries small and firm most of the time, but showed small follicles in December, 1949, January, 1950, and April, 1950, without any sign of oestrus. P.M.S. 15 c.c. on 12 and 13/1/50 had no effect. On 17/1/51 a firm follicle of 6 cm. was present in L.O. In next 3 days stilboestrol 10 mgm., 10 mgm. and 25 mgm. respectively given. Follicle was soft on 3rd day but regressed after that. Mare turned out with stallion on 13/3/51; was served and conceived within 2 weeks.
		3/12/49	5/12/49	3	Ovulation....	1st day....	Pregnant.....	A follicle in L.O. increased to 4 cm. on 2nd day, when it became soft and flabby, and ruptured within 24 hours.
1013 9 years	Shy. Irregular oestrous cycles							

INFERTILITY IN MARES CAUSED BY OVARIAN DYSFUNCTION.

TABLE 1 (continued).

No. and Age of Mare.	Breeding History.	Oestrous Periods in which Ovaries were Examined.		Duration of Oestrus in Days.	Termination of Oestrus.	Stage of Oestrus in which Mares were Mated.	Result of Mating.	Changes noted in Ovaries during Oestrous Periods.
		From.	To.					
1015 9 years	Normal foaling mare	(1) 3/10/49	18/10/49 <sup>†</sup>	16	Ovulation....	5th, 9th, 13th and 15th days	Pregnant.....	Follicles in both ovaries remained static the 1st 12 days. Stilboestrol 15 mgm. caused one in L.O. to grow to 6 cm. on 15th day. It ruptured within the next 24 hours. Follicle developed up to 4.5 cm. in L.O.; soft on 4th and ovulated on 5th day.
1016 12 years	—	(2) 27/11/50	1/12/50	5	Ovulation....	4th and 5th days	Pregnant.....	On 1st day one soft follicle 4 cm. in R.O. and 3 smaller firm ones in L.O. On 3rd the former was 6½ cm. and soft, while latter showed no change. Prolan 500 i.u. on 3rd day caused ovulation and conception within 24 hours, but oestrus continued another 4 days.
1018 14 years	Sent in with 1011 as a shy breeder in 1946, and has not foaled since	18/1/51	20/1/51	3	No ovulation	Not served	—	Like 1011 was also in anoestrus from 6/9/49 to 13/3/51 except for 3 days slight oestrus. Small follicles appeared in 1949/50 season but did not ovulate nor produce oestrus. Follicles of 6-6½ cm. on both ovaries on 18/1/51. Stilboestrol 15 mgm., 15 mgm. and 25 mgm. on these 3 days ineffective. Follicles regressed. Mare turned out with stallion on 13/3/51 and conceived within 2 weeks.



TABLE 1. (continued).

No and Age of Mare.	Breeding History.	Oestrous Periods in which Ovaries were Examined.		Duration of Oestrus in Days.	Termination of Oestrus.	Stage of Oestrus in which Mares were Mated.	Result of Mating.	Changes noted in Ovaries during Oestrous Periods.
		From.	To.					
1072 born 8/11/46	Maiden mare. In 1949-50 season she was in anoestrus from 30/11/49 to 18/4/50, and again from 21/4/50 to 6/10/50. After that cycles became regular	(1)	23/11/49 30/11/49	8	No ovulation	Not served	—	Follicles in both ovaries grew to 3 cm. One in L.O. was soft on 3rd day, but finally all receded. Both ovaries were small and firm, with no sign of follicular growth. By 9th day follicle in R.O. had increased to 7 cm., then receded. Small follicles in both ovaries on 1st day; all regressed. Follicles of 4 and 5 cm. in both ovaries on 1st day failed to respond to stilboestrol 15 mgm., 15 mgm. and 25 mgm. on 3 consecutive days, and both receded. <i>Note.</i> —During these periods service was often attempted in vain, because vulva was never relaxed and intromission of penis was not possible. Mare turned out with stallion on 13/3/51 and conceived within 3 weeks.
		(2)	18/4/50 20/4/50	3	No follicles, no ovulation	Not served	—	
		(3)	6/10/50 24/10/50	19	No ovulation	Not served	—	
		(4)	1/12/50 4/12/50	4	No ovulation	Not served	—	
		(5)	18/1/51 20/1/51	3	No ovulation	Not served	—	
1096 born 3/1/47	Maiden mare. Had very irregular oestrous cycles the previous season	9/10/50 31/10/50	23	Ovulation....	1st and 20th days	Pregnant.....	One follicle developed in each ovary. By 10th day that in R.O. was 3½ cm. and firm. The left was 6 cm. and firm. Nine days later the right one was 6 cm., softish and pitting, while the left was receding. Stilboestrol 10 mgm. that day caused ovulation in 24 hours, but oestrus continued another 3 days.	

INFERTILITY IN MARES CAUSED BY OVARIAN DYSFUNCTION.

TABLE 1 (continued).

No. and Age of Mare.	Breeding History.	Oestrous Periods in which Ovaries were Examined.		Duration of Oestrus in Days.	Termination of Oestrus.	Stage of Oestrus in which Mares were Mated.	Result of Mating.	Changes noted in Ovaries during Oestrous Periods.	
		From.	To.						
1112 born 5/1/47	Maiden. Erratic cycles in 1949-50 season when she failed to conceive to 4 services. In anoestrus from 17/1/50 to 28/10/50	(1)	10/11/49	14/11/49	No ovulation	Not served	—	Follicle in L.O. grew to 4 cm., was soft on 3rd day, but then regressed. Follicles in both ovaries attained only 1.5 cm. and then regressed again. A large somewhat soft follicle of 6½ cm. in L.O. failed to respond to 10 mgm. stilboestrol on the 1st day, and receded.	
		(2)	1/12/49	14/12/49	No ovulation	Not served	—	Follicle grew rapidly in L.O., was 7 cm. on 5th day, soft and pitting on 6th, ovulated on 7th day. A small follicle of 3 cm. in R.O. remained static.	
		(3)	28/10/50	31/10/50	No ovulation	1st and 4th days	No conception	No conception	Follicle grew rapidly in L.O., was 7 cm. on 5th day, soft and pitting on 6th, ovulated on 7th day. A small follicle of 3 cm. in R.O. remained static.
		(4)	17/11/50	25/11/50	Ovulation....	4th and 6th days	Pregnant.....	Pregnant.....	Follicle grew rapidly in L.O., was 7 cm. on 5th day, soft and pitting on 6th, ovulated on 7th day. A small follicle of 3 cm. in R.O. remained static.
1404 7 years	Never foaled. Erratic oestrus in 1949-50 when she was served 5 times without success	(1)	6/12/49	10/12/49	No ovulation	2nd and 5th days	No conception	Soft follicle of 3½ cm. in L.O. static for 4 days; then receded.	
		(2)	4/3/50	7/3/50	No ovulation	Not served	—	Mild oestrus caused by small firm follicle of 3 cm. in L.O. which failed to develop. Mare then went into anoestrus for 10 months.	
1418 born 22/3/48	Maiden.....		29/4/50	16/10/50	Ovulation....	118th, 127th, 165th and 168th days	Pregnant.....	This filly remained in oestrus throughout the winter. This was not due to persistence of a few follicles, but to a succession of follicles which developed and receded in both ovaries. Stilboestrol 10 mgm. on 127th day had no effect. On 170th day a large follicle 6½ cm., soft and pitting was present in L.O. while all others in both ovaries had receded. This ruptured within a few hours, and next day the ovary was painful, and mare resented palpation.	

TABLE 1 (continued).

No. and Age of Mare.	Breeding History.	Oestrous Periods in which Ovaries were Examined.		Duration of Oestrus in Days.	Termination of Oestrus.	Stage of Oestrus in which Mares were Mated.	Result of Mating.	Changes noted in Ovaries during Oestrous Periods.
		From.	To.					
1595 born 26/11/48	Maiden mare.....	(1)	3/6/50	30/9/50	No ovulation	Not served	—	Started oestrus in mid-winter with small follicles of up to 2½ cm. in both ovaries. These receded and were succeeded by others but all failed to mature. Mucosa of vagina and vaginal cervix always pale and dry. A large follicle was present in each ovary on 2nd day. Both developed gradually up to 6 and 7 cm., and both ovulated on 8th day.
		(2)	18/10/50	26/10/50	Ovulation....	Not served	—	
1734 11 years	Normal foaling mare	(1)	8/11/49	14/11/49	No ovulation	Not served	—	Firm follicle in L.O. on 3rd day; remained static for 3 days; then regressed. A hard follicle in L.O. only developed to 2.5 cm. on 4th day; then receded. Small firm follicle in R.O. and a larger of 5 cm. in L.O. on 1st day. Latter developed rapidly, was 7 cm., soft and pitting 2nd day and ruptured within next 24 hours. Follicle in R.O. remained stationary and oestrus continued for 5 days after ovulation.
		(2)	1/12/49	5/12/49	No ovulation	Not served	—	
		(3)	21/12/49	28/12/49	Ovulation....	2nd and 4th days	Pregnant.....	
2005 8 years	Barren. No history of previous foalings Had been used as a polo pony	(1)	3/10/50	31/10/50	Ovulation....	22nd and 26th days	Pregnant.....	At first a follicle developed in L.O. and was 6 cm. on 21st day, and 7 cm. and soft on 25th day, but then regressed. Meanwhile the R.O. pushed up a follicle which was 7 cm., soft and pitting on 28th day. Still-boestrol 15 mgm. given on 22nd and again on 26th days. Follicle in R.O. ruptured on 29th day. Oestrus shown when 7-8 weeks pregnant. This was due to a large follicle of 6 cm. in R.O., which ruptured on the 4th day.
		(2)	20/12/50	23/12/50	Ovulation during pregnancy	Not served	—	

INFERTILITY IN MARES CAUSED BY OVARIAN DYSFUNCTION.

TABLE 1 (continued).

No. and Age of Mare.	Breeding History.	Oestrous Periods in which Ovaries were Examined.		Duration of Oestrus in Days.	Termination of Oestrus.	Stage of Oestrus in which Mares were Mated.	Result of Mating.	Changes noted in Ovaries during Oestrous Periods.
		From.	To.					
2029 14 years	Barren. Sent in with history of being a shy breeder	9/12/50	15/12/50	7	Ovulation....	7th day (8-12 hours after ovulation)	No conception	Follicle in R.O. grew up to 6 cm. on 5th day. Stilboestrol 10 mgm. next day caused ovulation within 24 hours, ovary showing well-marked fossa which was painful on 7th day. The only service was given 8-12 hours after ovulation and did not result in conception.
2030 10 years	Barren and shy breeder	9/12/50	15/12/50	7	Ovulation....	4th and 7th days	Pregnant.....	R.O. developed a follicle which was 6 cm. but firm by 6th day. Stilboestrol 10 mgm. same day caused ovulation in 24 hours. Ovary was painful and had a well-defined crypt on 7th day.
2079 16 years	Sent in as a shy breeder	18/1/51	25/1/51	8	Ovulation....	3rd, 5th and 7th days	No conception	On 2nd day R.O. had a firm follicle 6 cm. By 5th day this was 7 cm. but still firm. Stilboestrol 25 mgm. caused slight softening of follicle by 6th day. Another 25 mgm. stilboestrol had no effect by 7th day. Luthormone 1,000 i.u. that day was followed by ovulation within 24 hours.

TABLE 2.

No. and Age of Mare.	Breeding History.	Out with Stallion in Camp.		Result of Pregnancy Examination on 18/5/51.
		From.	To.	
<b>GROUP A WITH STALLION 2039.</b>				
1011 (12 years)	See Table 1.....	13/3/51	9/4/51	Pregnant: 8 weeks.
1018 (14 years)	See Table 1.....	13/3/51	9/4/51	Pregnant: 8 weeks.
1072 (4 years)	See Table 1.....	13/3/51	9/4/51	Pregnant: 6-7 weeks.
1404 (7 years)	See Table 1.....	24/1/51	9/4/51	Pregnant: 3½ months.
1734 (11 years)	See Table 1.....	19/2/51	9/4/51	Pregnant: 2 months.
1932 (12 years)	Shy breeder.....	24/1/51	9/4/51	Pregnant: 6 weeks.
1973 (8 years)	Barren.....	24/1/51	9/4/51	Pregnant: 3½ months.
2003 (9 years)	No history of previous foaling. Had been used as a polo pony. Showed no oestrus from 8/1/51 to 13/3/51	13/3/51	9/4/51	Pregnant: 2 months.
2004 (8 years)	Same as 2003.....	13/3/51	9/4/51	Pregnant: 2 months.
2060 (10 years)	Reputed shy breeder. Showed no oestrus from 17/1/51 to 13/3/51	13/3/51	9/4/51	Pregnant: 6 weeks.
2075 (10 years)	Barren.....	24/1/51	9/4/51	Pregnant: 6 weeks.
2029 (14 years)	See Table 1.....	24/1/51	9/4/51	Not pregnant.
<b>GROUP B WITH STALLION 2010.</b>				
382 (11 years)	Normal foaling mare.....	19/2/51	17/5/51	Pregnant: 6 weeks.
387 (12 years)	Shy breeder and barren.....	19/2/51	17/5/51	Pregnant: 2½ months.
1010 (13 years)	See Table 1.....	19/2/51	17/5/51	Pregnant: 3 months.
1013 (9 years)	See Table 1.....	19/2/51	17/5/51	Pregnant: 2½ months.
1452 (18 years)	Normal foaling mare.....	19/2/51	17/5/51	Pregnant: 3 months.
1479 (18 years)	Reputed shy breeder.....	16/3/51	17/5/51	Pregnant: 6 weeks.
1481 (18 years)	Sent in as shy breeder.....	19/2/51	17/5/51	Not pregnant.
1482 (17 years)	Shy breeder.....	19/2/51	17/5/51	Pregnant: 5 weeks.
1488 (16 years)	Barren.....	19/2/51	17/5/51	Pregnant: 8 weeks.
1558 (18 years)	Barren.....	19/2/51	17/5/51	Not pregnant.
1939 (12 years)	Barren.....	19/2/51	17/5/51	Pregnant: 3 months.
1943 (14 years)	Barren.....	19/2/51	17/5/51	Pregnant: 3 months.