CONCEPTION FAILURES IN CLINICALLY NORMAL COWS

by

JORGE ANTONIO VILLAR

D. V. M., Buenos Aires University
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Major Professor

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INTRODUCTION

The perpetuation of the species is the ultimate aim for being of all organisms and involves the reproductive processes that may vary in their complexity and in the mechanisms employed. The two organisms involved in sexual reproduction are highly specialized. The degree of such specialization is the result of the complex synchronization of reproductive events, which, as a rule, follow predictable, well regulated patterns.

It is this complex of interlocking systems which ensure reasonably successful tune of the function of the sex mechanism.

The processes of reproduction occasionally become less efficient because one of the hormonal, neural or humoral links becomes impaired, or they function out of phase, disrupting the sensitive mechanism of synchronization that underlies most reproductive events. Nalbandov, (1958), p. 1.

This report deals with one of the most intriguing problems in the field of animal reproduction, the one better known as the "hard to settle" or "repeat breeding cow". To quote:

> One of the most perplexing and discouraging problems in a herd in which most of the animals breed normally, is the infertile cow with a normal estrous cycle and no apparent pathological changes. In our experience, approximately 5 to 8 per cent of the animals of a given herd may be placed in this category. (Kiesel and Dacres, 1959)

Another paragraph taken from Chambers (1948) expresses the importance of the condition, and also a fairly good description of the symptoms involved.

> Up until three years ago (1945), a large percentage of sterile cows, four to ten years of age, coming in heat regularly but still not conceiving, were either sent to the

butcher or were continually being bred. These cows were all individuals that had had two or more calves, had little if any pathology (that I could detect) and, I repeat again, they were all coming in heat with regularity.

There exists, in general, agreement that the "repeat breeding" cow must--regardless of the other symptoms involved--have repeated heat without settling. Most authors reviewed here accept a range between 2 and 4 or more infertile services. Nevertheless, Casida (1961) maintains that, provided the semen quality and insemination circumstances are optimal, an animal may qualify as a repeat breeder upon a single return from service.

However, for the sake of joining the majority of the opinions, we shall define the repeat breeding cow as the one who fills the following conditions.

1. Bred several times without success.
2. A minimum of one calving, but not over 10 years of age.
3. Regular ovarian cycles, after breeding.
4. No purulent discharge or physical abnormalities of the genital organs detectable by palpation per rectum.
5. History of reduced fertility, with undiagnosed cause.

In order to avoid unnecessary repetitions of excellent reviews which have appeared in classical texts related with the subject (Roberts, Malbandov, Salisbury and Van Denmark, etc.), this report will deal mostly with some aspects of the problem that are the object of study at the present. The role of specific infections (vibrosis and brucellosis), trichomoniasis, improper handling of semen, failure of detection of heat in the females, or any other cause whose importance has been undoubtedly established, will be overlooked for the sake of brevity.

FAILURES OF FERTILIZATION

When analyzing the causes for "repeat breeding" cows, Roberts (1956a.), p. 387, attributed 35 to 45 per cent of the cases being due to failure of fertilization due to obstructions of the oviduct, abnormalities in ovulation, and to defects or deficiencies in the ovum or spermatozoa.

Obstruction of Oviduct

Obstruction of the oviduct including adhesions between the ovary and burse, account for 3 to 4 per cent of the cases. However, Rowson (1942) found bursal adhesions of varying degrees in 13.3 per cent of 296 cows, and Simon and McNutt (1957) found hydrosalpinx, chronic interstitial salpingitis and pyosalpinx in 10% of 154 cows.

Failure of Ovulation

According to Roberts' data, failure of ovulation occurred in about 3 to 5 per cent of "repeat breeders". Nevertheless, he cited Hancock's work (1948) in which 69 per cent of 58 cows studied had ovulated by the day after estrus and 31 per cent by the second day after estrus.

More recently, Van Rensburg and DeVos (1962) reported that defective ovulation was the most common causal factor in the production of the "repeat breeding" problem. They found that of a total of 536 estrus periods observed in 118 Friesian and 161 Afrikaner cows, ovulation was normal in 396 and failed in 140

(26.2%). In the 140 cases ovulation was delayed in 92 (66%) and anovulation was the termination in 47 (34%) with many of the affected animals revealing a tendency to s how recurrent ovulatory failures. To support their view, they cited LaGrange (1958) who compared the results obtained from insemination in the first 12 hours of estrus with the results obtained 13 to 30 hours after the beginning of estrus in 1, 130 first insemination and 380 repeat inseminations. LaGrange showed that in normal fertile cows there was no noteworthy difference in conception between early (58.6%) and late (60%) inseminations, whereas with the repeat breeders there was a very significant difference in that only 47 per cent conceived as a result of insemination in the first 12 hours as against 70 per cent in those bred later in estrus or even after cessation of heat. In their own report, Van Rensburg and DeVos note that when 18 cows in which the follicle did not rupture within 24 hours after insemination were bred only once, none conceived. In 51 cows where repeat inseminations were given 24 hours after the first insemination the conception rate rose to 2.7%. They also cited Autrup and Rasbech (1951) who inseminated 293 cows about 48 hours after the end of the estrus in the post estrus hemorrhagic period, with the result that 87 (29.6%) conceived. Trimberger (1948) reported the different percentages of conception rate at different hours before and after the estrus period, with failure of any ova to become fertilized 48 hours after the end of estrus. Trimberger attributed the failure of fertilization to the failure of the ova to survive longer than a few hours after ovulation.

Aging of Ova and Spermatozoa

According to Roberts (op. cit.), p. 388, aging of ova affecting fertilization rate occurs only in cows bred artificially, as cows bred naturally would refuse to stand for the bull when well out of estrus. Salisbury and Van Demmark (1961), p. 483, adds that "the natural period of the receptivity of the female for the male would tend to confine the aging effect to time limits readily exceeded in artificial insemination."

The available data do not preclude the possibility, however, that aged eggs may be fertilized by sperm of varying ages. The important fact is that aged eggs that had been fertilized seem to lose the ability to continue normal embryonal development. Blandau and Young (1939) showed that guinea pig eggs lose their ability to be fertilized gradually and that those eggs that had been fertilized did not develop to the stage where they could form embryos capable of completing development. As late as 14 hours after ovulation, 56 per cent of the eggs could still be fertilized; 20 hours after ovulation 31 per cent of them could still be fertilized, but the percentage of abnormal pregnancies was 73 and 90 per cent, respectively. Barrett (1948) supported that data in cows showing that the conception rate was high and the early embryonic death rate low when heifers were inseminated within 2-4 hours after ovulation. But as more time elapsed between ovulation and insemination, the conception rate decreased and the early embryonic death rate increased rapidly. The percentage of fertile ova (75%) corresponded with the percentage of normal embryos at 21-35 days, when the

insemination was performed at 2-4 hours from ovulation. The fertilization rate was 60 per cent but the per cent with normal embryos was only 31 per cent at 21-35 days, when insemination was performed 9-12 hours after ovulation. The fertilization rate decreased to 40 per cent when insemination was made at 18-20 hours after ovulation, and the percentage with normal embryos at 21-35 days was only 17.

According to Hancock (1962), chronological age of spermatozoa can refer to the interval from the time of their release from the seminiferous tubule or to age from the time of ejaculation, the second case being referred to the time spent in the female tract of mated females. In artificially inseminated females it also includes the duration of storage "in vitro". Fertility of bull semen declines with age of storage "in vitro". He cited Salisbury and Florchinger (1961) who have found a decrease in the DNA content of spermatozoa with storage time. Salisbury et al. (1961b.) subjected to the Feulgen technique a subsample of sperm on each of the storage days selected (2, 3, 5 and 10 days). Analysis showed a marked progressive decrease in the Feulgen-DNA content of the stored spermatozoa. The loss of 30% of the initial DNA at the end of 5 days' storage was highly significant statistically. The authors support the data that apparent embryonic mortality resulted from the use of similarly aged spermatozoa for artificial insemination. Hancock states that this finding raises the possibility that decline in fertility might be directly related to loss of DNA from living spermatozoa.

Defects or Deficiencies of Spermatozoa and Ova

Inability of the sperm to fertilize is apparently the most common cause for failure of fertilization in normal cows and probably also in "repeat breeders". Failure of fertilization occurred for no apparent cause in 24 to 38 per cent of the "repeat breeding" cows. The importance of spermatogenesis of bulls has been pointed out by Knudsen (1958), indicating that important abnormalities of spermatozoa are overlooked by present routine techniques of evaluating semen. He observed that in normal bulls, spermatogenesis proceeds with great precision with the chromosome number being constant. Bulls with acquired disturbances in the spermatogenesis always show the same damage in the spermatogenic epithelium irrespective of the cause. The nucleus becomes pyknotic and while the chromosomes may divide the nuclei does not, resulting in a nucleus with twice the number of chromosomes. If the sample of semen shows a high content of these abnormal nuclei, it indicates a reduction in the number of spermatogonia and a definite sterility is developing. Of the abnormalities described, i.e., stickiness of the chromosomes, multiple spindle formation and chromosome aberrations, the latter seems to be the more important. In this type, there is a disturbance in meiosis due to a segment in one of the chromosomes becoming inverted (inversion) or due to an exchange between two chromosomes (translocation). When inversion occurs, homologous genes match each other and spermatozoa formed in this manner appear entirely normal and can fertilize ova, but the zygote will lack a balanced gene complement and the

consequence will be death of the embryo. In other cases, half of the sperm will have a normal gene complement and half a chromosome with the same inversion of the father; the progeny will be one half fully fertile and the other half will have lowered fertility. The same disturbances appear when translocations occur.

According to Bearden et al. (1956) it seems highly probable that the same kind of aberrations found in spermatogenesis occurs in oogenesis, and the structural changes in the chromosomes would be expected to have the same frequency in both sexes. Thus ova which are nonfertilizable are probably produced, due to the hereditary defects.

In another series of studies headed by Leuchtenberger (1960) concerning the relationship of deoxyribonucleic acid (DNA) to the fertilizing ability of the sperm of man and bull, it was reported that in fertile males the DNA content of spermatozoa shows a constant haploid value, with very little variation from male to male. In contrast, the DNA content of spermatozoa of infertile males did not show a constancy but frequently a marked variation, with DNA values significantly lower than the haploid amount. It was also variable from sperm to sperm, from sample to sample and from individual to individual.

Since the sperm analyzed had been selected for study only by its normal appearance--sizes of sperm nuclei were nearly identical for the fertile and infertile males--the difference in the DNA content among the groups was considered of great importance. It was also demonstrated that the low DNA content is not a permanent feature, but may vary from sample to sample for the same infertile

male, and even revert to a normal content. For this reason it was considered that a DNA analysis of a single seminal specimen which gives a normal DNA value is not indicative that the individual belongs to the fertile group. However, in a series of 108 specimens derived from 18 infertile males, only 15% of the samples had had a normal mean DNA value, even though the counts, morphology and motility were normal. The author states that although the causes for the faulty DNA synthesis are unknown, there are indications that hormonal factors may play a role in that a threshold quantity of hormones are required to guarantee the synthesis of a normal quantity of DNA in the primary spermatocytes. In other lines of observations, it is shown that the capacity of a sperm to fertilize an egg is closely linked to the exact haploid DNA content. While working with the clam "Spisula Solidissima", she found that when eggs are exposed to sperm with the normal haploid DNA amount, a selective advantage for penetrating the egg can be observed for the sperm carrying the normal DNA amount as compared to sperm with low DNA quantity. Furthermore, all activated eggs contained sperm with the normal haploid DNA amount, while the great majority of the inactivated eggs revealed sperm carrying deficient amount of DNA. These results might indicate that DNA deficiency in sperm may be responsible for at least one type of male infertility.

MATERNAL ENVIRONMENT

Maternal environment is considered to be one of the master keys for the enlightening of the factors affecting the "repeat breeding" cow. There are two different points of view of long

standing on the defects of the maternal environment. The first one sustains that the sterility is due to the presence of a low-grade, non-specific infection in the female genital tract. The second affirms that sterility is of a functional type and involves endocrine controls and various physiological functions of the genital tract.

Histopathological Findings

In order to fit the definition of repeat breeders, the presence of a specific or non-specific infection has to be subclinical, that is to yield no symptoms detectable by rectal palpation.

Several workers have studied the histopathological changes produced in uteri of repeat breeders, seeking evidence that the changes in the endometria might have been produced by the presence of infection.

Hallmen (1921) observed that endometritis was one of the common, yet more difficult conditions to diagnose, and that the accumulation of inflammatory cells, predominantly lymphocytes, in the tunica propria in close association with the epithelium could be readily observed in many sections from uteri that showed little or no gross pathologic changes. These accumulations were quite consistent in sections from infertile animals showing only a mild chronic metritis or infertility with no other symptoms.

Simon and McNutt (op. cit., 1957) utilized tissues collected from 87 repeat breeders which were killed 3 days after an experimental breeding and 22 repeat breeders which were killed 20-35 days after service. They detected abnormalities in only 20% of

the cows and these were minimal. Eleven cows had dilated or cystic, and in no case were more than 20 per cent of the glands cystic. When these cows were compared with virgin heifers, a part of them showed a moderately increased number of lymph follicles, 16 showed heavy infiltration with eosinophils also found occasionally among the control heifers. Number and distribution of polymorphonuclears within the endometria were not significant. Alterations within the oviducts that would account for the repeat breeding were found in 15 of 154 cows. These alterations included hydrosalpinx and chronic interstitial salpingitis or pyosalpinx.

The bacteriological examination of the cows showing abnormalities was negative. The authors were of the opinion that this factor would account for a small amount of evidence of endometritis found. They concluded that the endometria of repeat breeders did not differ greatly from that of apparently normal heifers, and that endometritis was not a major cause of repeat breeding.

In a similar study, Moss et al. (1956) utilized 55 cows from the U.S.D.A. herd at Beltsville and 22 cows from the Wisconsin University herd. The animals were killed at various stages of the estrus cycle or during the early stages of pregnancy. The abnormalities encountered involved the presence of distinctive nodules in the endometrium or distinct changes of the uterine glands and periglandular connective tissue. The types of abnormalities found varied between individuals, since nodules were not found in any of 25 animals with good breeding qualities, but appeared in 27 per cent of the animals with breeding difficulties. Encapsulation of mucous glands of the endometrium was the most common

abnormality noted and and these occurred only in cows from the Beltsville herd. However, the occurrence of nodules alone and of cystic glands alone was not statistically significant when related to breeding performance. A very high significance was found for the simultaneous occurrence of encapsulated glands and breeding difficulty. The presence of lymphoid nodules--uncommon in the bovine endometrium--did not appear to interfere with normal pregnancy up to the 36th day, since normal embryos were found in seven of the nine animals in which nodules were found.

Modified glands in uterus were also found more frequently in one group of sterile animals than in normal ones by Cupps and Laben (op. cit.). These glands were found in one case of uterine inflammation, but were not seen in another. Nor were they more frequent in cows with a history of uterine inflammation than in cows without it. This data suggests to the authors that uterine inflammation was not the cause of the modified glands, especially since no other evidence of uterine inflammation was apparent.

Easley et al. (1951) found little correlation between the histopathological or gross changes and the bacteriological findings, in 28 repeat breeding cows from 4 to 13 years of age. Although pathogenic bacteria were present, the reaction by the endometrium was slight. Trotter (1961) reported little discernible reaction on the part of the endometrium. He found that congestion, edema, leucocytic and glandular activity and condition of the epithelium often were similar in uteri from which bacteria were obtained to those in which no bacteria were found.

Bacteriological Findings

The question of whether or not there are microorganisms in the normal bovine uterus has not been completely solved. Analyzing the data, Elliott at K. S. U. (1961) suggested that the normal uterus could easily become contaminated at partuirition, which would account for the presence of bacteria at early days post-partum. In a survey in 110 post-partum bovine uteri he found a distinct correlation between length of the post-partum period and the presence of microorganisms. Eighty-five per cent of the uteri examined from 0-15 days post-partum contained microorganisms, and only about 10 per cent of the uteri at 60 days post-partum contained organisms.

The same divergence of opinion exists as to whether or not the presence of bacteria is an exclusive finding in the repeat breeder and if so if its presence is in direct relation with the "repeat breeding" condition.

Beaver et al. (1921) were of the opinion that the "diphtheroids" organisms were responsible for much of the sporadic infertility occurring in cattle. However, they indicated that the theory of infection did not explain all the cases of low fertility studied.

Hatch et al. (1949) cultured cervical smears from the genital organs of 53 infertile cows and reported the cultures negative in 26 cases and positive in 27. Positive cultures contained organisms of the Corynebacterium (bovis and renale), "diphtheroids" and gram-positive cocci groups. They could not catalogue by the case as to whether or not the microrganisms found were pathogenic or

saprophytic. They concluded, however, "That bacterial infection, either clinically apparent or not apparent may be responsible for many cases of infertility".

Cultures made by Lindley and Hatfield (1952) from uterine washings of infertile dairy cows yielded 26 separate species of bacteria with micrococci and members of genus Neisseria being the most prominent. However, the number of cows cultured was not listed and the number that were positive or negative to bacteria was not given. The authors concluded that the bacteria present could cause or aggravate the infertile condition.

A comparison of the bacteriological results obtained from samples taken from cows with a normal breeding history with those obtained from cows with a history of difficult breeding was performed by Easley et al. (op. cit.). Bacteriologically sterile samples were obtained from 56 (39%) of 146 normal cows in contrast to 8 (20%) of 40 repeat breeding cows sampled. The predominate organism, Staphylococcus pyogenes was found in 26 per cent of the normal cows and in 95 per cent of repeat breeders.

Gunter et al. (1955) conducted a bacteriological study of 260 uterine and cervical swab samples obtained from 106 dairy cows at successive heat periods. The survey included 201 samples from 92 normal breeders and 59 samples from 14 repeat breeders. Of the samples from normal breeding cows, 33 per cent were sterile while only 5 per cent of those from repeat breeders were sterile. The Streptococcus species, Micrococcus pyogenes strains, Corynebacterium and other diphtheroids species were shown to be predominantly saprophytic strains in normal breeders, while in the

repeat breeders the species in that genera were primarily pathogenic strains.

Nevertheless, Kiesel and Dacres (op. cit.) reported that 17 out of 24 uteri of infertile cows were negative for bacteria. They isolated pathogenic bacteria, Vibrio fetus and Staphylococcus aureus from 2 uteri, and nonpathogenic bacteria, Staphylococcus albus and a nonhemolytic streptococci from the remaining five uteri.

Gibbons et al. (1959) reported that although the percentage of bacterial isolation from infertile cows was no higher than those from fertile cows, the ratio of pathogens to nonpathogens was much higher in the infertile cows. However, when a correlation of presence of bacteria to future breeding was made, it was found that the presence of bacteria in the genital organs before breeding had no relation to subsequent fertility: the conception rate was essentially the same, 1.79. An unexpected result was that the preponderance of genital disease and sterility occurring after breeding was found in the group of cows negative for bacteria and also in the group of cows harboring nonpathogens. Cows harboring pathogens that were treated with an antibiotic (400 mg. Tetracycline) conceived at a higher rate than the general average of all cows. Cows harboring pathogens and not treated bred at approximately the same rate as the general average. Thus, there was no difference in conception rates among control cows harboring pathogens, treated cows and bacteriologically negative cows.

Viral Findings

Infertility due to viral infection was reported by Millar (1955) as associated with a vaginitis, edematous endometritis and embryonic mortality. He cultured the filterable agent in eggs and was able to reproduce the condition with third passage egg material by intravaginal, but not by intravenous inoculation. He concluded that the virus might enhance the virulence of potentially pathogenic organisms already present.

Lukert (1961) at Kansas State University was unable to find any evidence of viral infection in 47 uterine samples cultured in embryonated eggs and on bovine kidney tissue. The samples were from cattle with known breeding problems, cattle that had aborted, normal post-partum and pregnant cows. Although viral agents were not demonstrated, Lukert thought it possible that latent viral infections might be involved in the etiology of early embryonic deaths, and were difficult to demonstrate as they would not manifest themselves until they were unmasked under certain conditions, such as pregnancy. He also suggested the possibility of an inapparent infection in some of the animals studied. He cited Giroud et al. (1950), who showed that an inapparent infection with Rickettsia prowazekii in rats caused a high incidence of embryonic mortality, 40.9% of fetus absent after inoculation of the organism. However, the rickettsial organism could not be demonstrated in the reproductive tract, and the infected pregnant rats showed no signs of infection. The only means of detecting the organism was by recovering it from the peritoneal fluid and

demonstrating agglutinins in the blood stream.

Low Grade Infections

The low incidence of pathological changes noted in absence of generalized inflammation of the uterus, does not preclude the possibility of the existence of infections, which with little or no visible effect on the maternal tissues, might yet affect the gametes or the embryo within the lumen of the genital tract.

The existence of such infection of a low-grade type has been widely discussed by many workers. Uterine infusions with antibiotics have been given credit of increasing the proportion of repeat breeders carrying normal embryos. This has attributed to the elimination of the low-grade infection. However, more recent papers do not seem to substantiate this conclusion.

Chambers (op. cit) claims excellent results from the use of penicillin for treatment. A hundred thousand to 500,000 units--preferably 200,000 units--in from 10 to 50 c.c. of sterile water instilled into the uterus constitues his usual treatment. He says that to the best of his knowledge this type of treatment has been 90 per cent effective. In his opinion, the majority of the cows that come in heat regularly without an excessive amount of pathology, and do not conceive after several breedings, are suffering from a low-grade uterine infection that can be corrected with penicillin.

That intra-uterine injection of an antibiotic increases the conception rate in repeat breeders was also reported by Hasley

et al. (op. cit.). Their study was done in 377 cows classified as normal, covering 624 pregnancies from 963 inseminations (conception rate: 64.8%), and in 80 repeat breeding cows covering 59 pregnancies from 471 inseminations, with a conception rate of 13.6 per cent. They reported that 29% of repeat breeders conceived within two services following a rest period of 2 to 5 months, compared with 56% that received an intra-uterine injection of antibiotic during one of the heats occuring in the sexual rest period. In another trial involving 22 repeat breeders that were inseminated in the heat period following the treatment with antibiotics, the conception rate was 59% after 2 services; but when the intrauterine injection was made the day following service, 65% of the cows conceived from that service and 15% more on the following service making a total of 80%. They concluded that low-grade infections were the cause of the impaired fertility.

Lindley (1956) used intra-uterine therapy in cows with an indication of infection detected by clinical examination of the genital tract. He used streptomycean sulfate, 1 Gm and penicillin sodium or potassium 200,000 to 500,000 units. Those conceiving at the concurrent estrus equaled 64.3% for the treated, and 33.3% for the controls. He also favored the theory of the infection of nonspecific types.

Huber (1957) tested the efficacy of uterine infusions during the breeding-rest period at 20 to 45 days post-partum. He based his evaluation on the number of breedings per conception. When a breeding-rest infusion of terramycin was given, the average conception rate was reduced from 2.18 to 1.46, or a difference of

.72 breeding per cow. According to the author the treatment definitely improved the conception rate, since only 54 breedings were required for 37 conceptions in the group receiving the infusions (37 cows), whereas 80 breedings were required for 37 conceptions in the untreated group.

The failure of a combination of antibiotics as a treatment for repeat breeding cows was reported by Ulberg et al. (1952). Such a treatment consisted of an injection via the cervix into the lumen of the uterus, of a mixture consisting of 0.25 Gm. of intravenous aureomycin, 1.0 Gm. dihydrostreptomycin, and 100,000 units of crystalline penicillin in 125 ml. of sterile distilled water. The percentage of normal embryos found after 34 days post-service was 34.8 for the treated animals and 56.0 for the non-treated animals. The difference was not significant when tested by the Chi-square method. However, the fact that 14 out of 25 cows which received no treatment had had normal embryos, and that 10 of these resulted from only one more breeding (these cows had received an average of 6.8 inseminations prior to the experiment) was interpreted by the author that clinically normal repeat-breeding cows can settle without treatment.

Roberts, S. J. (1956b.) arrived at the same conclusion after evaluating the use of four types of uterine infusions in the treatment of 539 repeat-breeding cows. In 101 cows with an average of 3.74 services (range 2 to 12) before treatment with Preservisal (isotonic Ringer's solution and dextrose), 39 (38.6%) conceived on the first service after treatment and 56 (55.4%) conceived the first and second services after treatment.

Thirty-three cows (33%) were sold as sterile.

In 133 cows with an average of 3.84 services (range 2 to 8) before treatment with an iodine solution and sexual rest for one estrus, 68 (51.1%) conceived on the first and 85 (63.9%) conceived on the first and second services after treatment. In 45 cows with an average of 3.7 services (range 2 to 9), before treatment with tyrothricin and sexual rest for one estrus, 17 (37.8%) conceived on the first service and 23 (51.1%) conceived on the first and second service after treatment. Eighteen cows (40%) were sold as sterile. One hundred and sixty cows with an average of 3.65 services (range 2 to 10) before treatment were infused with penicillin and streptomycin in a water and oil emulsion, in distilled water, or in a sodium sulfonamide solution. Seventy-four (46.3%) conceived on the first service and 96 (60%) conceived on the first and second services. Forty-six (28.7%) were sold as sterile.

When the author compares the figures with the percentages of cows that conceived at each service from the first to the seventh or eighth service in a data on nearly 50,000 cows, he found that data presented by most other authors reporting on intrauterine infusion were similar to the author's data on untreated cows when a large number of cows are compared. He adds that none of the recommended treatments for infertile or repeat-breeder cows show any significant improvement over the data with no treatment at all. According to the author, the chances of a cow's conceiving on the third service is about 50%, on the fourth service 40 to 45%, and on the fifth and sixth services 30 to 35 per cent.

Another evaluation of uterine infusion as a treatment for repeat breeders was made by Hjerpe (1961). He found no statistically significant differences when comparing the conception rate after treatment with 300,000 units procaine penicillin G and 1 Gm. of dihydrostreptomycin sulfate in 10 c.c. of 0.9% saline solution, with the conception rate of control repeat breeders infused with 0.9% saline solution. Twenty-six out of 38 antibiotic treated cows became pregnant (68.4%); 20 out of 33 controls were pregnant (60.6%).

Kiesel and Dacres (op. cit.) also failed to obtain any improvement in the breeding efficiency of infertile cows, when four kinds of antibiotics infused mostly 4 to 24 hours after service were tested.

Attempts to Reproduce the "Repeat Breeding" Condition

Keisel and Dacres (op. cit.) attempted without success to induce lowered breeding efficiency by inoculation of heifers with organisms isolated from the genital organs of repeat breeders. Three groups of heifers were infused with Streptococcus pyogenes, Staphylococcus aureus or Diplococcus pneumoniae. The infused heifers conceived at the same rate as a group of controls, and the organisms could not subsequently be recovered from the cervical mucus. When the same organisms in both cultures were placed in the uterus of the same heifers as soon as the fetal membranes were dropped after calving, there was no appreciable difference in subsequent conception rates between the bacteria-inoculated animals (3.2 services) and the controls (3.0).

Menge et al. (1961) inoculated the uterus of eight virgin heifers with selected bacteria and bacterial toxins, and subsequently tested them for fertility. The inoculum for 2 heifers consisted in live cultures of Escherichia coli, Staphylococcus aureus and Corynebacterium renale (app. 30 million cells). Another 2 heifers received the same inoculum plus 5 ml. of S. aureus toxin in the first and second estrus cycles; these 2 heifers and the remaining 4 animals received 250 million organisms each from live cultures of E. coli and S. aureus and 1.0 ml. of S. aureus toxin. Following inoculations the heifers usually showed purulent vaginal discharge and swelling of the cervix and uterus which persisted for 3 to 4 days. The heifers were artificially inseminated at the first estrus following the last inoculation; six conceived on first service and 2 required a second breeding. The high fertility obtained failed to indicate any carry-over effects from the earlier history, which has sometimes been postulated to explain the clinically normal repeat breeder.

Stage of Estrus Cycle Related with Infections

Several workers have gathered information relative to testing the effect of the stage of the estrus cycle in relation with intrauterine infection.

McDonald et al. (1952) injected both bacterially sterile epididymal semen and ejaculated semen to three rabbits in estrus and in four ten-days pseudopregnant rabbits; the right horn served as a control for the left horn, since there is no direct

connection between the lumens of the 2 horns in rabbits. Twenty-four hours post-insemination, they found the uterine horns of the estrus rabbits devoid of any gross or microscopic lesion, while in the four pseudopregnant rabbits, inoculation of bacterially contaminated semen produced a gross pyometra. This indicates that under the influence of estrogen, the uterus appears to be capable of ridding itself of irritants including bacteria, but when under the influence of progesterone, the uterus has greater difficulty in responding in such a manner. Hansel and Asdell (1957) suggest that estrogen results in the proliferation of the terminal blood supply to the bovine uterus. They base this on the fact that in the rabbit estrogen is known to increase the blood flow through the uterus which may strengthen uterine resistance to infection. In addition estrogen may stimulate uterine contraction, thereby increasing the flow of mucus through the cervix. To the contrary, progesterone increases glandular activity and "uterine milk" formation which supplies not only an essential nutrient for the developing egg but also one most suitable for bacterial growth.

To answer the question of whether the action of estrogen was responsible for the absence of the inflammatory response in the estrus uterus or whether a high progesterone level was the causative factor in the development of pyometra in pseudopregnant uterus, Black et al. (1953a.) compared the inflammatory response to bacterial inoculation in pseudopregnant and in ovariectomized rabbits with and without treatment. They found that pyometra was produced in untreated pseudopregnant females and in spayed animals

treated with progesterone. It was not produced in untreated or estrogen treated ovariectomized rabbits. They also demonstrated that the difference in response to infection was not the result of a purely mechanical elimination of the irritant material from the estrus uterus, when they failed to induce pyometra in the ligated horn of an inoculated estrus uterus of rabbits.

Black et al. (1953b.) compared the inflammatory response of the bovine endometrium between normal and repeat-breeding cows. Semen was deposited in the uteri of 3 estrus and 3 twelve-day luteal phase virgin heifers. No response was observed in the estrus females at slaughter 24 to 36 hours later, while the uteri of the luteal phase heifers contained pus. Then they made a direct comparison inseminating four virgin heifers- repeat breeder pairs, seven to 14 days post-estrus, each pair from the same semen sample. Histological studies revealed an actue endometritis accompanied by pyometra in the normal heifers. The repeat breeders showed considerable less cellular exudate, suggesting that the reaction was of a more chronic character as if their uterus had been subjected frequently to such stimuli. For these workers, the relative non-responsiveness seen in the repeat breeders might indicate an endocrine dysfunction (progesterone deficiency) underlying their lowered fertility. However, Roberts (1956a.), p. 390, suggests that the difference in response is due to prior or existing uterine inflammation with an increased bacteriostatic activity of the endometrium which provides a ready defense against introduced infections, rather than to a deficiency of progesterone.

Lamming and Rowson (1953) confirmed the results found by Black et al. when showed that in every case where cows were inseminated during estrus with semen containing C. pyogenes, the uteri were sterile 2 days later, while 9 out of 11 cows inseminated during the luteal phase had had pyometritis. They conducted a second experiment in which they inseminated 9 ovariectomized cows with infected semen. Four were the controls (only infected semen); 2 cows were injected intramuscularly with 25 mg. stilbestrol, and inseminated at the following day (estrus) with infected semen. The remaining 3 were given 25 mg. stilbestrol, followed by a daily injection of 50 mg. progesterone during 4 days and then were inseminated with infected semen. Progesterone injections were maintained until slaughter. In the controls 3 developed pyometra, but bacteria were absent 2 days after infection; the 2 animals receiving stilbestrol had sterile uteri which appeared normal and healthy while those receiving progesterone all had a gross infected pyometritis.

In order to test their ability to produce and control pyometritis in the cow, 6 cows in the luteal phase were inseminated with a culture of C. pyogenes and 2 days later the existence of pyometritis was confirmed. On the same day the corpus luteum was expressed manually and cows were killed two days after heat: in no case was pyometra present and bacterial swabs of the uteri at slaughter were sterile. Then they tested the ability of stilbestrol to overcome the infection in the presence of a corpus luteum. After producing pyometritis following the same procedure, 10 cows were then treated with stilbestrol at various dosage

levels: 25, 50, 75 and 100 mg. In 2 out of 3 in the 75 mg. levels, and all 3 at 100 mg. levels had sterile uteri even though a heavily infected pyometritis was present 3 days previously.

This seems to prove that the susceptibility of the uterus to infection is related to the relative levels of progesterone and estrogens, and that the effect of one may be overcome by an excess of the other.

The authors report that there are certain indications that the highly resistant effect during estrus might be a general effect rather than localized in the uterus. They cited vonHaam and Rosenfeld (1942) who showed that antibodies produced in rabbits after injections of pneumococci were at a higher level when oestrone was given than otherwise; Foley and Aycock (1944) who found that a very much lower percentage of mice died after injection of streptococci when stilbestrol was given than when no stilbestrol was administered. Also Moberg (1952), who observed that there was a considerable increase in blood leucocyte numbers in cattle during estrus as compared with blood obtained during the luteal phase.

In a later report by Black et. al. (1954) presented evidence to support the theory that the endometrium of the repeat breeder possesses a great bactericidal activity (or lower susceptibility to infection) than that of the first service animal. They found that bactericidal or bacteriostatic activity was practically non-existent in pseudopregnant rabbits. They based their conclusions on comparisons made of the number of bacteria recoverable from the uteri of estrus, spayed and pseudopregnant rabbits in-

oculated 24 hours before with known concentrations of Escherichia coli. In experiments designed to compare the uterine bactericidal activity of luteal phase cows of normal and impaired fertility, a similar pattern was observed, although the lowering of the defense mechanism in the luteal phase cow was not as intense as in rabbits. However, the authors noted that whether this difference in response was the direct cause of the difference in fertility or only an associated phenomenon, could not be determined with present techniques.

Based on these observations Casida (op. cit.) concluded that the repeat-breeder uterus, rather than carrying a low-grade infection, may be even better able to cope with bacterial invaders than the uterus of the normal animals, and, that the more effective uterine defense mechanisms in the repeat breeder may actually indicate a functional basis for the lowered fertility, that is to say a lesser action of progesterone. The progesterone action might involve a lowered production, a lowered sensitivity of the uterus, or a more rapid loss of the hormone between the source and the target organ.

Infections and Early Embryonal Death

Several papers have been published dealing with the approximate date at which the early death of ova would be more common. According to Tanabe and Almquist (1953), 55.5% of 36 repeat-breeding heifers scheduled to be slaughtered at 34 days came into estrus before 30 days after their last breeding. In Tanabe and Casida's report (1949) about 50 per cent of 53 repeat-breeding

cows came back into estrus prior to 32 days. Roberts (1956a.), p. 390, cited Bearden's report (op. cit.) in which 8 of 26 heifers bred to low-fertile bulls came back in estrus by 28 days, 7 of which were between 17 and 20 days.

Laing (1949) concluded that a certain percentage of fertilized ova die before the tenth day of the cycle with the cow coming in heat at the regular cycle interval, while in others the zygote dies after the middle of the cycle and the return to estrus may be delayed.

Hawk et al. (1955a.) reported that in repeat-breeding cows most embryonic deaths occurred between 16 and 34 days. They recovered normal embryos from the uteri of 29 (58%) of 50 repeat-breeding cows slaughtered 16 days after the first day of heat and 14 normal embryos (28%) from 50 repeat-breeders slaughtered at 34 days. The estimated embryonic death from 16 to 34 days was 51.7%. They concluded that the majority of embryonic deaths during that period appeared to occur by 25 days, since 77.8% of the cows which did not have a normal embryo at 34 days, had either returned to heat or had a quiet ovulation by the end of 25 days.

To test the possibility that infectious agents in the uterus were responsible for the embryonic deaths found in those studies, Hawk et al. (1958) undertook another experiment to determine if the uteri of clinically normal repeat-breeding cows contained microorganisms at 16 days of pregnancy. They utilized 69 cows with an average of 7.2 services per conception. Embryos were recovered from 30 out of 57 anatomically normaly cows, and 27

of the 30 uteri were bacteriologically sterile. In the other 3, Candida Krusei was isolated in two and Brucella abortus in the third. Organisms were isolated in only eight uteri (11.6%) of the 69 repeat breeders. Embryos were not recovered from 27 of the anatomically normaly uteri containing no pus. They concluded that it seems to be little evidence that infectious agents play a major role in the early embryonic death.

ENDOCRINE FINDINGS

Pituitary Gland Function

Attempts have been made to establish a difference in anterior pituitary gland function which might be responsible for the repeat-breeding condition.

The possibility that lactogenic hormone might be deficient in repeat breeders was investigated by Kiddy et al. (1956), with the assumption that lactogenic hormone is responsible for the maintenance of corpora lutea in cattle as has been demonstrated in rats and sheep. Comparisons were made of the relative lactogenic potency of the pituitaries collected 16 days post breeding from 10 repeat breeders and 10 first service dairy cattle. The acetone desicated pituitary powder was tested for its stimulating action on the pigeon crop-gland. Each pigeon received an injection of repeat breeder pituitary gland on one side and of first service pituitary gland on the other side of the crop-gland. Analysis of the weights of stimulated crop-gland areas failed to show any significant difference between the two. Cupps and Laben (op. cit.), could not find any differences in the content of

follicle-stimulating and luteinizing hormones between the pituitaries from these two kinds of animals.

Corpus Luteum Function

Effects of Exogenous Progesterone. Different experiments have been conducted in order to test the possibility that deficiency of progesterone, either as a result of decreased luteal function or of the regression of the corpus luteum could furnish an explanation for the different behavior between repeat breeders and first service animals. It was theorized that the embryo might be maintained in the absence of the corpus luteum by progesterone therapy, provided that the regression of the corpus luteum or a subminimal level of progesterone was responsible for the death of the embryo.

Herrick (1960) found that an injection of 500 mg. of progesterone (repositol) given at the time of service increased the conception rate. He reported that 35% of 20 repeat-breeding heifers conceived at first service compared with 5% of the controls. Dawson (1954) found that 47% of the treated animals settled as compared to 17% of the controls. Johnson (1955) treated 20 females having had from 4 to 9 infertile services with a respositol progesterone type and 16 conceived at first breeding (80%). In another trial, Johnson et al. (1958) used a total of 139 normal cows. When 53 were treated with a repositol-type progesterone, 27 (67.9%) conceived to a first service. The progesterone injections were administered on the 2, 3, 4, 6 and 9th days after breeding. When 17 females were given another type (*) of

(*) "Delalutin", E. R. Squibb and Sons, New York, N. Y.

progesterone intramuscularly on the second day after breeding, 13 conceived at first service (76.5%). Controls that did not receive progesterone conceived to first service for a breeding efficiency of 42% (29 out of 69). The conception rate of the combined treated group was 1.38, and 1.85 for the controls. The difference was significant at the .05 level.

On the contrary, Wiltbank et al. (1956) using only repeat-breeding cows, found no significant differences in the proportion of treated and untreated cows with normal embryos 34 days after breeding. They administered progesterone at different dosages (50 and 200 mg.) starting 3 days after the beginning of heat and continuing until the corpus luteum started to regress or if it did not regress, until 34 days after heat.

They also found in some animals that exogenous progesterone was unable to maintain an embryo beyond the time of corpus luteus regression; therefore, they concluded that something other than the supply of progesterone was the limiting factor in the maintenance of the pregnancies. In addition the fact that they found functional corpora lutea in a few control animals accompanied by dead embryos and degenerate membranes is a failure as evidence that the deaths of the embryos were caused by regression of the corpus luteum. It would rather seem that the deaths of the embryos were the cause of the regression of the corpus luteum.

Hansel et al. (1960b.) also failed to demonstrate any advantage of the use of progesterone, when used at the dosage of 20 mg., subcutaneously, in 36 cows with an average of 3.1 services without conception, and 28 controls.

Measure of Activity of Corpus Luteum Function. Foote et al. (1959) studied the endocrine activity of corpora lutea from first service and repeat-breeder heifers by measuring weight, total progesterone content and proportion of functional luteal cells. Each heifer was allowed to have at least one normal estrus cycle and on the 14th day after heat, the corpus luteaum was removed through a supravaginal incision, leaving both ovaries intact. After the next heat, a second corpus luteum was removed on the 14th day. The differences were statistically insignificant. This lack of differences in the progesterone levels of normal and repeat-breeding cows was later confirmed by Erb and Stormshak (1961).

Zimbelman et al. (1961a.) reported that corpora lutea from heifers on day 14 of the estrual cycle had a higher percentage of functional cells than heifers in the early stages of pregnancy.

Changes in the progesterone levels of corpora lutea during the estrual cycle have been reported by Mares and Casida (1960). They studied the corpora lutea from heifers at 7, 9, 11, 13, 15 and 17 days following estrus. Progesterone concentration increased from 25.8 ug. per gm. at day 7 to 44.7 ug. (per gm. of fresh luteal tissue); then decreased to 7.0 ug. per day at day 17.

A normal drop in progesterone levels of the corpora lutea during early pregnancy was reported by Zimbelman et al. (1961b.) They found that progesterone and total progesterone (sum of progesterone plus 4-pregnene-20 beta-01-3-one, a progestational activity compound) were significantly lower on day 28 and 42 of pregnancy than on day 14, the decrease being gradual instead of

occurring more during a part of the interval. Another drop was reported to occur between day 14 and 28 of pregnancy, but this time only in progesterone content but not in the other progestational compound.

Modification of C. Luteum Activity with Oxytocin. Another approach to the problem of the repeat breeder was the experimental modification of corpus luteum function.

One of the bases of the experimental procedure was established by Armstrong and Hansel (1959), when they discovered that daily injections of oxytocin during the first seven days of the estrus cycle inhibited the formation and function of the corpora lutea, causing heifers to return to estrus at the eighth to tenth day of their cycles. But they also discovered that oxytocin injections were incapable of inducing estrus in hysterectomized heifers, a result which suggested to them that the uterus might be involved in the oxytocin response.

Hansel and Wagner (1960) confirmed these results when oxytocin injections were given during the first third of the estrus cycle (days 3-6). But they also got the same results inserting small catheters through the cervix into the uterus (during estrus or on the first day after estrus), and filling the attached balloon with water in order to dilate the uterus; or by inoculating larger than normal amounts of raw semen--or the sediment obtained by centrifugating raw semen and preputial fluids--into the uterus of heifers at estrus. The two latest methods served to dilate the uterus, which, in turn, inhibited the secretion of a luteotrophic hormone from the anterior pituitary. With respect to the

oxytocin injection, the authors suggest that it may provoke uterine contractions or may have a direct action on inhibit anterior pituitary luteotrophin secretion. They claim to have proved in that way, that the uterus plays an important role in regulating estrus cycle length and luteal development in the bovine, and that this mechanism might be an important factor in determining whether or not an embryo survives during the critical first 30 days of pregnancy.

Staples and Hansel (1961) took advantage of the discovery that oxytocin inhibits partially or completely corpora lutea function, in order to investigate the relationship between progesterone level in the corpora lutea and embryo survival in 70 dairy heifers at 15 days after insemination. Forty-three heifers were each injected subcutaneously with 7 U. S. P. units of oxytocin per 100 pounds of body weight daily for 14 days, commencing on the day following the initiation of estrus. The remainder were controls; all were slaughtered on the morning of the 15th day. Data obtained indicated that embryo development could proceed normally when the level of progesterone in the corpus luteum was above the threshold value, and that it fails to occur at subthreshold value. They did not recover embryos from heifers having less than 100 ug. of total progesterone in their corpora lutea. Two out of three heifers having degenerate embryo had less than 100 ug. of progesterone. Sixteen of 24 treated heifers without embryos had had luteal progesterone levels below 100 ug. Therefore, they concluded that a threshold level of progesterone of the order of 100 ug. is necessary for embryo survival at the 15th day.

Staples et al. (1961) considered the effect of varying luteal progesterone levels on the rate of embryo development. They recovered embryos 15 days after insemination from 74 per cent of untreated heifers and from 42 per cent heifers treated daily with oxytocin. The embryos from the two groups did not differ in total length, embryonic disc size or mesodermal development attained. They confirmed their former work in which no embryos were recovered from animals with less than 100 ug. of total progesterone in their corpora lutea. They concluded that the results provided little evidence to support the theory that the rate of embryo development in the early stages is proportional to the rate of progesterone secretion in the corpus luteum. A suggestion is made that normal embryo development occurs when the level of progesterone production exceeds the threshold level represented by approximately 100 ug. of luteal progesterone. This was supported by the finding of normal embryos at the same stage of development in heifers having as little as 108 ug. and as much as 883 ug. of total luteal progesterone.

Modification of Corpus Luteum Activity with Progesterone and Estrogens. Modification of corpus luteum development and function were also tested by ways of injecting progesterone and estrogens. Spies et al. (1958) demonstrated that progesterone treatment from the 10 to the 25th day after mating caused a regression of the corpora lutea in hysterectomized as well as in pregnant gilts and indicated that progesterone acts independently of the uterus. Loy et al. (1960) studied the effect of injected progesterone and estrogen on the progesterone content, proportion of luteal cells

and weight of corpora lutea in open heifers. One mg. of progesterone per lb. body weight in a single injection on day one of the cycle caused significant differences in all three variables between treated and control corpora lutea on day 14. The same hormonal treatment on day 5 in another group of heifers caused the same significant differences, except in corpus luteum weight. When heifers were given 250 mcg. of estradiol-17 beta per day from day one through day 13 of the estrus cycle, significant differences were found between treated and control corpora lutea for weight and proportion of functional cells, but not for progestogen concentration.

The results obtained appear to be explainable on the assumption that formation of the corpus luteum and maintenance of its function are dependent upon optimal rations of estrogen and progesterone. The authors suggest that the mechanism by which imbalances of the ovarian hormones damage the corpus luteum involve presumably altered pituitary gonadotropic secretion, mainly a lack of circulating LH by a blockade of LH release, produced by the action of the progesterone injections. Ray et al. (1961) suggest that progesterone might suppress production rather than suppress release of LH. They agreed with the findings of Loy (op. cit.) since they found that pituitary FSH and LH potencies were significantly higher in control animals than in the group treated with Repositol progesterone.

The unbalance between the ovarian hormones produced a decreased secretion of "uterine milk" which is considered necessary for nourishment of the developing blastocyst.

Greenstein et al. (1958) and Wiltbank et al. (1961) also have shown that injections of estrogen induced early regression of the corpus luteum in the cow. In addition, the latter workers found that injections of gonadotrophins, following injection of estrogen, prevents early regression of the corpus luteum; thus, they postulated that the regression of the corpus luteum induced by estrogen was the result of a deficiency of gonadotrophin, probably LH. Works were completed to determine the effect of daily injections of human chorionic gonadotrophin on the maintenance of the corpus luteum and on embryonic survival in the cow. Daily injections of 1,000 I. U. of H. C. G. into five cycling heifers from the 15th day to the 26th day of the estrus cycle maintained the corpora lutea and lengthened the estrus cycle from 17.7 to 32.4 days. However, 39 bred heifers which received HCG from the 15th to the 35th day post-breeding did not show an increase in pregnancy. It was concluded that there exists a sufficient amount of evidence to prove that regression of the corpora lutea during the estrus cycle of the cow may result from a deficiency of gonadotrophins, presumably LH, because the early regression of corpora lutea produced in cows by estrogen can be stopped or reversed by injection of gonadotrophins, or maintained past normal time of regression by injection of HCG. The deficiency of LH and regression of the corpora lutea could be caused by a high level of estrogen in the mid-cycle.

IMMUNOLOGICAL FINDINGS

Antibodies against Sperm

Another possible aspect of repeat breeding from functional causes is immunological in character. It has been demonstrated that the spermatozoa of many animals are antigenic, stimulating the production of specific antibodies when injected into animals of the same or different species. The question arises if such antibodies may affect the fertility of the immunized animal. Conflicting reports are found in the literature on the question of whether female fertility can be reduced by immunization with sperm. The procedure generally followed in these reports has been to immunize the females with a sperm preparation and then to place them with fertile males to determine if and when they would produce normal young.

Kiddy et al. (1959) artificially inseminated female rabbits with rabbit semen that had been treated with immune serum produced in cattle against semen from several different male rabbits. When diluted rabbit semen was mixed with normal sera, fertilization was not affected. With high concentrations of immune sera fertilization was prevented but when lower concentrations were used, fertilization was possible. The females were killed at different stages after insemination to provide information on fertilization rate and embryo survival. The average embryo survival percentage for 58 immune-sera females was 38.2 compared to 53.1 for 59 normal-sera females. The loss of whole litters was more common in the immune-sera group (23 females out of 58) than it was in the normal-sera group (3 females out of 59). A significantly greater amount of embryonic deaths occurred in the immune-sera group.

Alfonso and Perez and Perez (1960) reported that intramuscular injections of semen into rabbits raised the agglutinin concentration of serum, and that when this exceeded 1 in 200, mucoagglutinins in the vagina were also present. These produced infertility in concentrations over 1 in 100. Similar mucoagglutinins could also be produced by daily insemination for 40 days and eventually serum agglutinins could also result from this stimulus. Such a positive sero-agglutination reaction has been detected at the rate of 0.5% in cattle, and only at low levels. According to the authors the short heat period of the cow reduces opportunity for the phenomenon to occur.

Menge et al. (1960) worked the problem with cattle, with similar results. Six heifers were inseminated with bull semen that had been diluted with antiserum from a heifer immunized against the sperm of the bull. Five heifers returned to heat an average of 21 days after breeding while the sixth had a degenerating embryo 42 days post-breeding. Two heifers bred with semen diluted with "normal" serum became pregnant.

In order to explain the unknown immune mechanism leading to infertility, Katsh (1960) proposed that any antigenic material delivered into the female tract during copulation is capable of inducing infertility. The fact that Austin (1951) had observed phagocytosis of sperm in the uteri of rats after copulation, together with his own observation of the same thing occurred in the guinea pig, suggested that phagocytic removal of sperm could be the mechanism whereby the tissues of the female reproductive tract manage the antigen (sperm). In this way the sperm

is carried to other parts of the body by the phagocytes and antibody production ensues. Katsh suggests that the antibody response seems to be increased by complexing of the antigens with lipopolysaccharide of bacterial origin and other agents, to form the hapten-antigen complex. However, he observed that sperm antigens are rather weak in the sense that a lasting immunization is not usually produced in the female. To maintain the infertile state it was necessary for the immunization to be sustained by continual mating. The animals became fertile again when coitus was not permitted for 2 or 3 months.

He pointed out the possibility that any part of the male or female reproductive tract that is antigenic may influence the fertility of the respective individual. Although he was unable to induce infertility in rabbits by injecting corpus luteum, he presumes the corpus luteum is an antigenic organ, as well as the placenta and maybe the endometrium. To emphasize the role of the proteins complex hormones, he cited Maddock et al. (1956) whose paper demonstrated that some of the women treated with FSH obtained from hog pituitaries developed antibodies not only to the exogenous FSH but also to the endogenous gonadotrophin with inhibition of ovarian activity being its consequence.

According to Casida (op. cit.), whether antibodies against sperm are built up in the genital tract in nature, or whether the sperm antigens are transferred from the tract into more intimate contact with the antibody-forming system (as postulated by Katsh), and the antibodies in turn carried back to the genital tract, has not been definitely established. Closely related possibilities

receiving attention at the University of Wisconsin are that embryonic death may result from certain blood antigen differences between mother and offspring or from pleiotropic action of genes for certain blood antigens.

Beta-globulin Type

Ashton (1959) concluded, from the results of matings between parents differing in beta-globulin type, that the chances of survival of the bovine embryo are affected by the beta-globulin genotype of the dams. It has been estimated that 12 per cent of the fetal loss in British cattle is due to dam-fetus beta-globulin incompatibility. In another report by the same author (1962) the breeding efficiency for matings between partners both homozygous at the beta-globulin locus was 57.98 per cent compared with 47.68 per cent for partners one or both of which were heterozygous. In another of the two different Australian regions tested, the comparable breeding efficiency was 62.93% and 47.24% respectively. In each region, the breeding efficiency with the homozygous bulls was about 4% greater than the mean for the region.

Ashton and Fallon (1962) used indirect methods to determine whether the former results were due to differential mortality of the embryonic genotypes, or to differences in fertilization efficiency. They found that an excess of heterozygotes are born since the homozygotes are less viable in the uterus than heterozygotes. However, they found also that mating between homozygous parents of like genotype have a greater chance of

achieving fertilization than matings between homozygous parents of unlike genotype. They concluded that the beta-globulin locus in cattle affects fertility both at fertilization and in the uterus.

GENETIC FINDINGS

According to Johansson (1960), many reproductive disturbances are apparently due to endocrine imbalance with the predisposition for such imbalance being, however, probably hereditary. The manifestation of such imbalance usually depends on the environmental factors, mainly stress of various sorts through under or over feeding, management, climatic conditions, diseases, etc. He stated that although the animal is protected in some extent against these factors, when the threshold of resistance is exceeded, the reproductive disturbances become phenotypically manifested. The genetic analysis of this type of reproductive disturbance is extremely difficult, not only because of the complexity of gene interactions and interactions between genotype and environment but also owing to the inaccuracy in the phenotypic measurement of the degree of the disturbances.

Inherited Predisposition

It is recognized that most of the variations in fertility of cattle from year to year are due to environmental influences, since little alteration in the genetic structure of a herd can occur in such a short time. The study of herds records, however, shows some evidence that there exists an hereditary

predisposition to high and low fertility. Differences in the fertility of cattle families have been recorded many times; one of the earliest reports is that of Kab (1937) involving 136 bulls, 1475 cows and 7106 calves. When the cows were divided into classes, according to the number of viable calves produced at a given age, the total incidence of fertility disturbances was 26.37%. When an analysis was made of the fertility of daughters of 22 bulls, considerable variation was found between the various families, which pointed to a genetic basis. High fertility in 35 families and low in 11 families could be observed in several generations.

Trimberger and Davis (1945) reported that, among 20 cow families, there was one with a significantly high service rate (2.92 services per conception) and 2 with significantly low rates (1.22 and 1.00). One family had had 6 sterile cows out of 16. They believed that this pointed to an inherited sterility. In another study made by Fett (1953) on the records of 50,000 animals of the Lower Bavarian Spotted cattle, the occurrence of a hereditary predisposition to high and low fertility was shown.

Jones et al. (1941) concluded--when the breeding records of the dairy herd of the Oregon Agricultural College were analyzed--that cows with the greater number of descendants usually bred more regularly, dropped more vigorous calves and bred to an older age. Much of the infertility in certain breeds and families was attributed to the inheritance of low fertility and closed breeding for some character other than good fertility.

Concerning the inheritance from dam to daughter, Pou et al. (1953) concluded in their study of the inheritance of breeding efficiency in the Beltsville herd based on data accumulated over a 30 year period, that the intraherd repeatability and heritability values were low. They added that if the number of services per conception, number of days from first service to conception, or the regularity of the occurrence of estrus are used as measures of breeding efficiency, then selection for improvement in breeding efficiency would not be very effective. This concept was shared by Trimberger and Davis (op. cit.) when they said that it was not possible to predict the breeding efficiency of the daughters from the breeding records of the dams (the comparison was made in services per conception).

Nevertheless, Spielman and Jones (1939) reported some evidence of inheritance for good fertility based on a correlation between the reproductive efficiency of foundation cows and that of their female descendants. Korkman (1947) reported that variation in the number of services per conception depends partly upon heredity, this being responsible for ten per cent of the variation in fertility of animals discarded because of sterility.

Wilcox et al. (1956) studied the records of a Holstein-Freisian herd and showed that daughters of dams with a lifetime production of 5 or more viable calves were themselves more fertile than the daughters of dams producing less than 5 calves. Their conclusions were based on results from 23 and 153 dam-daughter pairs in each group in which heritability of fertility was 0.30.

Inheritance, Conception Rate and Embryonal Death

The relationship of conception and embryonic mortality with inheritance had been extensively studied. In 1952, Laing stated that there was no conclusive evidence of an hereditary predisposition to early embryonic mortality, although Tanabe and Casida (op. cit.) had found differences between breeds, Guernsey cows having lower fertilization rates and higher embryonic mortalities than Holstein, an effect that was offset by a higher genital abnormality rate in the Holstein breed.

According to Hawk et al. (1955b.), the sire has a highly significant effect on the rate of embryonic death in the females to which he is bred. When the embryo was a result of non-related parents, the percentage of death rate was lower (19.8) than for the inbred embryos (27.3). When the dam was a daughter of non-related parents, her sons had an estimated 19.2 per cent of embryonal death, whereas for inbred dams the percentage was 26.4. Although the differences were not statistically significant in either case, the trend was that inbreeding of either embryos or dams, particularly the latter would increase embryonic mortality.

These results were confirmed later on by Mares et al. (1961) who reported the pregnancy loss as being affected significantly by system of mating in cows. In this study, higher conception rates were found in the system of mating in which the potential embryo was outbred than those in which the potential embryo was inbred. Furthermore, pregnancy loss (until 152 days) was greater in the system of mating in which the dam was inbred than those in

which the dam was outbred. They concluded that there was an indication that inbreeding of the potential embryo is more detrimental to the fertilization process and/or early embryonic death than the inbreeding of the dam, whereas later embryonic and fetal loss were affected more by the inbreeding of the dam than by the inbreeding of the embryo or fetus.

Malbandov (op. cit.), p. 259, cited data obtained from rats suggesting that females unable to conceive after a single exposure to males produce daughters that require 1.5 heats before conceiving, and that, by the fifth generation, their offspring require 3.5 heats before becoming pregnant. In the original population 92 per cent of the females conceived after one exposure, but five generations later only 33 per cent of the daughters of the original repeat-breeder females (8 per cent) conceived at the first opportunity. He stated that although this sort of data is not available in domestic animals, and that if the data were applicable to them, caution in retaining hard-to-settle females would seem indicated.

NUTRITIONAL FINDINGS

Different Planes of Nutrition

Variable effects of feeding levels upon the number of services required per conception have been obtained among different experiments. In a review by Reid (1960) he mentioned the experiment initiated at Cornell in 1948 in which the primary objective was to determine the effects of quantity of nutrients provided

from birth to the time of the first calving, upon the reproductive performance in cows. When using three different planes of nutrition, low, medium and high, they found that the number of services required for the first pregnancy was the same regardless of the early-life plane of nutrition, with none of the groups requiring consistently more or less services than did the others for subsequent services. However, the results of other experiments, especially those conducted in New Zealand indicated that heifers fed a high-energy intake required more services per conception than did heifers on a low plane of nutrition. Furthermore, heifers reared on a high plane of nutrition conceived as well as their low-level mates when their feed intake was reduced to the maintenance level for approximately 1 month prior to mating and much more readily than when the high intake of feed was maintained. He also cited a Danish experiment in which heifers were reared to the time of the first calving, again on three different levels of energy intakes. The proportion of the animals allotted to the experiment which became sterile at some stage of their total life were 27.6, 29.0 and 50.9 per cent, for the low, medium and high levels, respectively.

Trace Minerals

Manganese deficiency was incriminated by Hignett (1941) as being associated with ovulatory disturbance, mainly delayed ovulation with the ovum not being liberated until five to seven days after estrus. He found that this occurred in certain herds in Britain as well as in Switzerland and New Zealand. Hignett

suggested that heavy applications of artificial fertilizer, including lime, might tend to lock up the manganese in the soil, rendering it unavailable for the plant and livestock.

Bentley and Phillips (1941) found that heifers raised on low manganese rations were slower to exhibit estrus and were slightly and consistently slower to conceive upon breeding. Ovaries were found to exhibit a low manganese concentration when the diet of the cattle contained only 7 to 10 p.p.m. of manganese. It is added that the deficiency might have an effect upon ovulation or the vitality of the ova.

That trace minerals have little if any direct relationship to the repeat-breeding cow problem was concluded by Bentley et al. (1951), when they studied the distribution of manganese and cobalt in the feeds fed the cows from problem herds and in the liver and ovaries of the cows. They found, however, a definite evidence of low or marginal cobalt intake associated with many of the problem herds. Low manganese concentrations while still within the normal range in the feeds of the farms resulted in low concentrations of manganese in the ovarian tissue.

However, Van Rensburg and DeVos (op. cit.) cited Tesink (1960) who compared the results obtained with artificial breeding in 19 herds in Holland, receiving trace mineral cakes, with that of 20 control herds, and found the conception rate to be 16 per cent higher in the former. The most marked improvement was seen in those herds in which the copper and manganese content of both the ration and the hair of the animals was low originally.

Miscellaneous

In another connection McClure (1961) mentioned a nutritional lactational stress causing an infertility problem characterized in its milder type by infertility in adults but not in first lactation cows, and in its more severe type by equally poor fertility in all ages and groups of lactating cows. The problem is reported to be temporary, most cases recovering spontaneously in 3 to 9 weeks, and seem to be associated with the failure of the cows to regain weight loss under the stress of increasing milk production during early lactation. It would seem unlikely that this would occur where 15 to 20 pounds daily of silage are being eaten in addition to pasture.

Marion (1962) reported recently that substances other than vitamin E, contained in wheat germ oil may be capable of beneficially influencing reproduction in repeat-breeder cattle. During a 3-year study, feeding of 2 ounces of wheat germ oil per week for 6 weeks resulted in a highly significant difference in conception in favor of the treated cows that had been bred four or more times before initiation of treatment, with respect to untreated controls.

SUMMARY

Clinically normal cows with failures of conception are also known as "repeat breeding" or "hard to settle" cows. They show a reduced probability of conception, although other factors are optimal. This paper attempts to review the findings regarding

the problem. The importance of failures in fertilization is discussed. Delayed ovulation appeared to be one of the important factors that had been overlooked. Aging ova is of relative importance only when artificial insemination is used. Studies on the processes of spermatogenesis indicate that some aberrations of spermatozoa capable of producing infertility of the male or death of the embryo are overlooked by present routine checking of sperm qualities. Aberrations in the processes of oogenesis are also suspected. Spermatozoa of normal appearance could lack a normal DNA value, this lack being related with the capacity of sperm to fertiline an egg. DNA content of spermatozoa appears to be lowered after 5 days' storage.

Histopathological findings in repeat-breeders' uteri could not be definitely related with the repeat-breeding condition. The scanty reaction on the part of endometrium is considered by some authors as being produced by a low-grade specific infection. However, the ability of uterine infusions with antibiotics to overcome the condition appears to be controversial. In addition, attempts to reproduce the repeat-breeding condition have also failed.

Deficiency of progesterone in repeat breeders has been postulated to explain the fact that the uterus of these cows during the luteal phase is more resistant to bacterial invasion than that of the first service animal. However, therapy with progesterone has not given clear-cut evidence of its value. Others judge that the difference could be due to a prior inflammation that had increased the bacteriostatic activity of the

endometrium of the repeat-breeding cow.

Much of the embryonic loss seems to occur between days 16 and 25 after breeding. Irritations of the uterus seems to provoke inhibition of the release of a luteotrophic hormone that theoretically maintain the functional corpus luteum in the cow. Embryo development seems to proceed normally when the level of progesterone is above a threshold value estimated at 100 ug. Early regression of the corpora lutea is suggested to be associated with a deficiency of LH caused by a high level of estrogen in the mid-cycle and the resulting imbalance of ovarian hormones affects the corpus luteum bringing about a decreased secretion of "uterine milk".

Antibodies produced experimentally have been suspected to produce fertilization failure and embryonic death. It was proposed that any antigenic material delivered into the female tract during copulation is capable of inducing infertility; the site and mechanism of the process appear unknown. Dam-fetus beta-globulin incompatibility is reported to produce fetal loss and the breeding efficiency appears to be influenced by the homozygocity or heterozygocity of the partners, with regard to the beta-globulin locus.

Genetic causes of lowered fertility have been demonstrated. In spite of the finding that its hereditability is relatively low, selection for other characters disregarding fertility appears to be a dangerous method. Inbreeding of the dam or fetus was reported to be detrimental to the fertility process and/or embryonic life.

High planes of feeding seems to increase the incidence of breeding difficulties, although the experimental results have been controversial. Trace minerals appear to be slightly associated; manganese deficiency seems to be related with delayed ovulation.

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CONCEPTION FAILURES IN CLINICALLY NORMAL COWS

by

JORGE ANTONIO VILLAR

D. V. M., Buenos Aires University
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Department of Surgery and Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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Approved by:

J. E. Mosier
Major Professor

Clinically normal cows with failures of conception are also known as "repeat breeding" or "hard to settle" cows. They show a reduced probability of conception, although other factors are optimal. This paper attempts to review the findings regarding the problem. The importance of failures in fertilization is discussed. Delayed ovulation appeared to be one of the important factors that had been overlooked. Aging ova is of relative importance only when artificial insemination is used. Studies on the processes of spermatogenesis indicate that some aberrations of spermatozoa capable of producing infertility of the male or death of the embryo are overlooked by present routine checking of sperm qualities. Aberrations in the processes of oogenesis are also suspected. Spermatozoa of normal appearance could lack a normal DNA value, this lack being related with the capacity of sperm to fertilize an egg. DNA content of spermatozoa appears to be lowered after 5 days' storage.

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