The most important genital diseases of cattle (control, treatment and the hygiene of semen collection)*

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Summary: This report reviews the infectious diseases (bacterial, protozoal and viral) of cattle which are transmissible by mating or insemination, with reference to pathological consequences, diagnosis and effective prophylactic measures.

While brucellosis, campylobacteriosis and trichomoniasis are generally under good control (although sporadic outbreaks may still occur), each disease has its diagnostic problems, which are discussed.

The difficulty of controlling IBR/IPV is emphasised.

Although chlamydiosis and mycoplasmosis cause only sporadic trouble, these infections should not be disregarded.

Health risks associated with embryo transfer are discussed.

Hygienic precautions to minimise the health risk of contaminated semen (with occasionally pathogenic bacteria) are discussed.

KEY-WORDS: Cattle - Europe - Fertility - Genital diseases - Disease control - Artificial insemination - Embryo transfer.

INTRODUCTION

Reproductive performance plays a fundamental role in the profitability of cattle herds, the main cause of economic loss as a result of infertility or subfertility being prolongation of the interval between calvings.

A great deal of research has been devoted to factors which may affect fertility (feeding, herd management, veterinary supervision, etc.), with the aim of reducing the proportion of cows culled because of infertility. The mean proportion may range from 3 to 7% (Rollinson, 1955; Withers et al., 1959; Boyd et al., 1961), coupled with a figure of 1 to 5% for abortions. According to Erb et al. (1980), a survey of the incidence of relative infertility and reproductive disorders showed that 10-30% of 2,960 lactations were affected, and 3-6% of the herd was culled for these reasons in a year.

As in many similar reports, this work (and a later report by the same authors published in 1981) classified the disorders by their clinical manifestations (ovarian

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cysts, endometritis, dystocia, placental retention, abortion, etc.) and not according to the pathogenic agents responsible for them. Bretzlaff et al. (1982) investigated the occurrence of reproductive disorders after calving, and listed the microorganisms found in these situations. However, the list comprised only the common bacteria which are occasionally pathogenic (Escherichia coli, Streptococcus, Corynebacterium, etc.) without indicating the involvement of specific pathogens responsible for the major diseases of the genital system.

Martinez (1983) studied reproduction in two large herds by means of weekly visits during two years, and found that 80% of animals were affected by reproductive disorders, among them puerperal endometritis at a frequency of 30-47% (and linked to true anoestrus). Poor reproductive performance arose chiefly from events occurring after artificial insemination, such as anoestrus after insemination and failure to conceive. Other disorders responsible for reduced fertility were placental retention, dystocia and ovarian disorders.

Such situations may give the impression that the major diseases of the genital system are no longer important, and it is true that many of them have been eradicated or at least brought under control by systematic vaccination, slaughter, restriction of movement, etc. The sporadic nature of those that remain does not lead to spectacular generalised occurrence, and their severe economic consequences are confined to limited outbreaks.

The disappearance (or reduced incidence) of the major diseases of reproduction has led to an improvement in fertility. However, this improvement has been matched by the growing importance of ordinary hygienic factors (bacteria which are often non-specific) and management factors (supervision, overcrowding, increased productivity) in the aetiology of reproductive disorders (Uwland, 1984). These factors alone are responsible to some extent for the aggravation and increasing importance of certain generalised diseases which may affect reproduction and are much commoner now than they used to be.

While it may be true to say that imbalance (or inadequacy) of physiological factors have assumed a more important role in reproductive disorders, and that such factors are more closely related to herd management than to infectious factors in the narrow sense, it must not be forgotten that such imbalance permits the establishment of strains of micro-organisms of enhanced virulence (such as endometritis caused by Pseudomonas aeruginosa), and may favour the development of epizootics of diseases which currently occur only sporadically (infection with IBR/IPV virus and Chlamydia).

There is no merit in separating diseases transmitted venereally from other diseases of the genital system, because certain diseases (e.g. brucellosis) are transmitted only exceptionally at mating, and most female cattle are no longer at risk of infection from natural service, this having been replaced by artificial insemination. Therefore, particular importance should be attached now to diseases transmissible by semen.

We shall now examine the specific features of these infectious diseases in males and females, and the contamination of semen by bacteria which may prove pathogenic under particular circumstances. Disease hazards associated with embryo transfer will be discussed, and finally ways of producing semen to diminish the risk of contamination with pathogens.
BRUCELLOSIS

Eradication campaigns have been introduced widely to control this disease, and they have been successful in many countries. Some, like Denmark, Great Britain, Netherlands and Romania are now free from the disease. In certain other countries less than 0.5% of cattle and 3% of herds may still be infected. Finally, regulations in Europe now usually specify that bulls admitted to artificial insemination (AI) centres must be negative to the agglutination test (AT) and/or the complement fixation (CF) test on blood serum. The bulls have to come from brucellosis-free herds.

The success of measures for the prophylaxis of brucellosis means that this is no longer an important disease of the genital system. Diagnosis has been standardised and is, in general, highly efficient. However, it should be noted that in Great Britain (Bell, 1984) and France (Parez, 1981) there have been problems of non-specific reactions to the serum agglutination test among bulls at AI centres, with AT titres between 30 and 80 IU and a negative CF test. Such reactions have appeared sporadically among bulls kept in isolation at AI centres for some time (1-3 years or more), and the reactions disappear after 1-8 months. This phenomenon does not seem to occur in countries which have been free from brucellosis for many generations of cattle (Denmark, Sweden). It would be necessary to examine the health records for the dam of such a bull to discover if the dam had been contaminated or vaccinated at a young age. Bell (1984) believed that the cause should be sought in an anomaly of the immune system, particularly of IgM.

There has been a report of positive serological reactions following contamination of foot and mouth disease vaccine with strain 19 vaccine against brucellosis (Parez, 1982).

CAMPYLOBACTERIOSIS

This is probably the most typical venereal infection capable of impairing herd fertility, for it is capable of causing both herd infertility and endemic infertility. It occurs throughout Europe and North America, but is at present only sporadic in Romania, Czechoslovakia, Netherlands, France, Cyprus and Great Britain.

The following species and subspecies are now recognised on the basis of serotyping and cultural and biochemical properties: C. fetus venerealis, C. fetus fetus serotype A, C. fetus fetus serotype B, C. fetus sputorum bubulus.

Their occurrence and role varies from country to country, and from one region to another. Bell (1984) reported that in Great Britain typical herd infertility is caused by C. fetus subsp. venerealis, while C. fetus subsp. fetus is associated with sporadic abortion, or in certain regions, herd infertility. Polak and Rysanek (1984) in Czechoslovakia found that C. fetus subsp. fetus occurred in herds of imported cattle.

Vanderlasche (1982) categorised the occurrence of different strains as follows:
- infertility: 90% caused by C. fetus venerealis A, 10% caused by C. fetus fetus B, rarely C. fetus fetus A;
- abortion only: C. fetus fetus A and B.
Campylobacter sputorum bubulus is considered to be a saprophyte, present in vaginal and preputial secretions, semen, fetuses and the placenta. Its presence may complicate laboratory diagnosis.

The serum of animals which have recovered from Campylobacter infection contains thermostable type O agglutinins, belonging to all three classes of immunoglobulins (IgG, IgM and IgA), and also local thermostable and thermolabile agglutinins belonging to class IgA. However, females which have never been in contact with the disease may possess type O agglutinating antibodies in their serum as a result of « infection » with the intestinalis biotype or the subspecies C. sputorum bubulus.

The infection is insidious in the male, being generally inapparent, without lesions, without symptoms and with no change in semen quality (even though the semen may become contaminated with Campylobacter). This makes detection difficult, particularly when coupled with the lability of the bacteria outside the host (rapid destruction upon exposure to light; destruction in 5 min at 58°C). Transmission takes place almost exclusively by mating or insemination (and also between male animals in close contact). It is impossible to establish a diagnosis on clinical grounds (prolonged oestrus cycles, repeated return to oestrus, increased number of inseminations required for each conception, prolonged interval between calving and conception, increased occurrence of abortion at 3-8 months of gestation). Moreover, under extensive husbandry and in beef suckler herds the disease will become more firmly established than in a dairy herd, because of the inevitable delay before a diagnosis is made. The bacteria remain confined to the vagina in only 25% of cows (which can have a normal pregnancy). More often the bacteria migrate along the genital tract, invading the body of the uterus and reaching the uterine horns 7-10 days after vaginal contamination. In about 15-20% of cows, infection of the oviducts results in salpingitis.

It is a self-limiting disease, which means that infected animals undergo spontaneous cure. The different classes of antibodies behave differently: IgA is formed in the cervix and vagina, where it may persist for some 10 months, while IgG is dominant in the uterus. These antibodies may prevent reinfection, but their presence does not mean that the female is no longer shedding Campylobacter.

Diagnosis depends on identification of the bacteria by direct microscopy or by culture.

Material and methods have been detailed in the norm laid down in March 1984 by the Norms Commission of the O.I.E., presented at the General Session of the Committee on 21-25 May 1984.

The most reliable type of sample is a preputial washing (made with broth medium or normal saline). Tedesco et al. (1977) compared three methods for taking samples from bulls and obtained the most uniform and precise results with the « scraping » technique devised for trichomoniasis by Sutka et al. (1969). The mucous membrane of the preputial sac is scraped with a grooved metal or plastic rod, and the method has given excellent results (Guérin, 1984). However, it should never be used in large herds unless disposable rods are available, one for each animal.

Another diagnostic technique for bulls is immunofluorescence (Clark et al., 1978), applied directly to fresh semen samples or to cultures prepared from them.
It gives precise, reliable and specific results, and is well suited for testing contaminated samples, even those containing few *Campylobacter*, after their life span outside the host has expired.

Proper preservation of a sample to be sent to a laboratory is difficult, since campylobacters cannot survive for more than 6 hours. Consequently, to ensure a reliable result from cultures, it is necessary to sow the culture on site, or to use a storage and enrichment medium if culture is to be performed later. In France, the « Laboratoire de Contrôle des Reproducteurs » uses SBL storage medium derived from that proposed by Gästrin *et al.* (1968). Other solid media (for swab samples) and fluid media have also been proposed for storage alone (Clark *et al.*, 1978), or both storage and enrichment (Bell, 1984). These techniques allow 95% of the bacteria to survive for 48 h, with a maximum life of 6-7 days (reduced in the presence of contaminants). A pure culture of *Campylobacter* keeps for about 3 weeks in such media. Storage and/or enrichment media should contain antibiotics (such as bacitracin, polymyxin, novobiocin or cycloheximide) to suppress contaminants.

The result is also influenced by the conditions of culture during incubation at 37°C, the best results being obtained with an atmosphere composed of 87% nitrogen, 5% oxygen and 8% carbon dioxide, and by using thiol medium plus antibiotics to control contaminants. The cultures are examined after 2, 3, 4, 5 and 6 days of culture — colonies usually form after about 4 days.

Serological tests on mucus and/or blood serum are of minor importance because of variable results and uncertainty concerning the source of the antibodies detected (perhaps being due to non-pathogenic strains).

Conventional therapy with antibiotics (streptomycin or erythromycin) has been abandoned because of false cures in both males and females, and the adverse effects on semen production by bulls. Vaccination of bulls infected with *C. fetus fetus* does not by itself lead to a certain and permanent cure. On the other hand, Fischerleitner (1984) showed that two inoculations of *C. fetus fetus* vaccine, a month apart, coupled with infusion of an intramammary formulation of antibiotics into the prepuce, or with intramuscular injection of streptomycin or intravenous injection of erythromycin, can produce a satisfactory bacteriological cure of breeding bulls, with little effect on semen production.

Such treatment always requires the frequent application of reliable diagnostic procedures to detect the bacteria, in order to confirm complete recovery before the bull is returned to service.

It is rare for females to be treated, except for vaccination, renewed annually. While the disease is self-limiting in individual animals, waves of recurrence tend to occur in a herd. A female may excrete *Campylobacter* for two years after infection.

Prophylaxis is based on isolating the breeding herd, rigorous examination of bulls brought into the herd, and the use of AI with semen from disease-free bulls. In general, bulls should be checked before being used for service (they should come from a herd known to be free from reproductive disorders, and a representative sample of animals should be negative to diagnostic tests) and subsequently twice a year.

In Great Britain, before a bull is used and while it is still in quarantine, it is routinely treated with an oily gel containing penicillin and streptomycin, introduced into the prepuce on three consecutive days (Bell, 1984).
The extensive use of AI coupled with rigorous application of technical and health surveillance, has practically eradicated the disease from countries previously heavily infected. However, occasional accidental infection still occurs, sometimes with rapid recurrence (perhaps due to infection with a new strain; Bell, 1984). It must be remembered that even a vaccinated bull can act as a passive vector after it has served an infected cow.

**TRICHRONGONIASIS**

Before the Second World War, trichomoniasis was considered in many countries to be a certain cause of bovine infertility as a consequence of endometritis, pyometra, early abortion and sterility. Transmission was exclusively by the venereal route.

In certain countries (Great Britain, Netherlands, France, Cyprus, Czechoslovakia) the infection has become a clinical rarity and an exceptional laboratory finding during the past 10-15 years. On the other hand, it remains a serious cause of infertility in other countries, particularly in dairy herds, poorly supervised herds and mountainous regions, wherever communal bulls are still used.

The disease has regressed considerably, or even disappeared, wherever breeding is well supervised, breeding animals are tested, and AI used on a large scale.

In the male, the protozoan parasite *Trichomonas foetus* colonises solely the mucosal crypts of the prepuce or penis, without visible signs of infection and without eliciting the formation of local antibodies, or specific agglutinins in the blood.

In the female, contamination occurs in about 25% of cases by natural service, and the parasite may remain in the vagina without penetrating to the uterus. However, when a cow is inseminated with contaminated semen, the parasite gains direct access to the uterus, causing death of the embryo either directly or indirectly (from endometrial lesions); the result is a return to oestrus. If fetal development is more advanced, abortion or fetal maceration may occur, with accumulation of pus (pyometra), while the corpus luteum remains active because the endometrial lesions have upset prostaglandin secretion. Secretion of local antibodies in the vagina may overcome infection, but is unlikely to protect against a subsequent reinfection.

Clinical diagnosis is derived solely from herd records showing a combination of many cases of return to oestrus, endometritis, purulent vaginal discharge, abortion and pyometra. Confirmation of the diagnosis usually rests on direct microscopic examination of preputial washings from the bull used in the herd, with culture of the parasite.

Diagnosis can also be confirmed by identifying the parasite in a purulent vaginal discharge and/or stomach of an aborted fetus.

However, such diagnosis, while easy to interpret, requires correct techniques for specimen collection (preputial washing during vigorous massage of the preputial sac), and very rapid examination at the right temperature (37°C), since the parasite dies in about 6 hours outside the host; at lower temperatures the movement of the parasite will be diminished or absent altogether. A mobile laboratory capable of doing the test and setting up a culture on site is essential to guarantee accurate and reliable results.
Serological diagnosis by the mucus agglutination test is unsatisfactory because of the poor immunogenicity of the trichomonas, even in the case of local antibodies.

The treatment of infected bulls by repeated applications of ointments containing acriflavine or trypaflavine has been largely superseded by systemic therapy with dimetridazole, given by mouth at 50 mg/kg once daily for 4-6 days. This usually cures most infected bulls and cows. A useful supplementary treatment for females is the injection of prostaglandin, which re-establishes a normal cycle in the uterine mucosa by its luteolytic action.

Prophylaxis and eradication of trichomoniasis require the abolition of venereal contamination by changing to AI. Of course, the bulls selected for AI have to come from a disease-free herd, and have to be quarantined before reaching puberty. During quarantine preputial washings should be taken on 2 or 3 occasions for examination for *T. foetus*. The bulls must not come into contact with untested cows used as teasers. These conditions are similar to those required for controlling campylobacteriosis.

In some European countries, such as Czechoslovakia, any animal found to be infected is not treated but eliminated (Polak, 1984), although in most countries dimetridazole is still used. Treated animals should not be used for breeding until 2 or 3 tests on preputial washings during the 2 months after treatment have proved negative. When a bull at an AI centre is found to be infected, its entire stock of semen should be destroyed as suspect from the date of the last negative test.

Trichomoniasis is a notifiable disease in Romania, and adult males and females have to be treated until repeated laboratory testing has shown that the infection has been eliminated. It is forbidden to move a bull over 6 months of age away from an infected premises, except for slaughter. This prohibition is lifted 3 months after the last known case of trichomoniasis has been cured.

**COITAL EXANTHEMA (IBR/IPV VIRUS INFECTION)**

This infection with bovine herpesvirus, leading to balanoposthitis in bulls and infectious pustular vulvovaginitis (IPV) in cows, has been recognised for several decades. It is a complex, baffling and changing disease, giving rise to much controversy, with prophylaxis proving difficult to devise and implement.

The virus is not selective for the genital tract, for it can become established in the respiratory tract, causing infectious bovine rhinotracheitis (IBR), and also in the brain and the udder. The various strains responsible for these different infections are serologically indistinguishable, but they are not altogether interchangeable, for a typical strain of IPV virus will affect only the genital system and udder, while an IBR strain may be capable of inducing respiratory, genital, mammary or cerebral lesions (Straub, 1978). Stresses and conditions of management are important in determining whether an outbreak occurs or not.

The virus is not very resistant, and it is transmitted directly by respiratory, venereal or bucco-genital routes, or indirectly by the contaminated hands of a farmer or a semen collector.

The infection may develop in both males and females without obvious clinical signs, or accompanied by non-specific inflammation, granulation tissue, vesicles
and ulceration (which may be due to secondary infection with non-specific microorganisms).

Specific antibodies appear soon after infection (8-10 days) and increase in titre until 20-30 days after infection, after which they decline and may even disappear. The course of this waning of antibody titre does not proceed smoothly and progressively, but is liable to fluctuations, with occasional unexplained resurgence. It can take 18-24 months for these antibodies to disappear. Even animals which have become serologically negative can remain latently infected, responding to some sort of stressor by resuming antibody formation together with virus excretion. We found that 4 bulls, serologically negative on many previous occasions, became serologically positive after they had been introduced into a herd free from the disease. The neutralising antibody titre in these bulls increased considerably after injection of dexamethasone, and they began to excrete virus in nasal secretion, sometimes with simultaneous excretion in the preputial secretion (Goffaux, 1984).

A single negative serological test does not rule out the possibility of the animal being infected.

The chance of virus excretion occurring during the course of time, and changes in the titres of serum antibodies have been investigated by Bitsch and by Straub.

In consequence, once an animal has become infected, it should be regarded as a latent carrier for life, and an animal which has given a positive serological test remains suspect for life (although how could one know that an animal at present serologically negative was positive two years ago?).

The principles of diagnosis are:

- attempted culture of the virus from specimens which may be contaminated with virus, such as semen (diluted to avoid the cytotoxic effect of spermatozoa), preputial washings, vaginal discharge;

- demonstration of virus neutralisation by serum from the animal under test (neutralisation test), as indicated by the cytopathic effect of a strain of IPV virus on cultured calf kidney cells. Every effort should be made to standardise the conditions under which the neutralisation test is carried out, for its sensitivity and reproducibility are governed by conditions such as serum dilution, time and temperature of heating the serum, and the right time to read the neutralising effect (Bitsch, 1970).

Given the extensive spread of the virus in the world and its economic consequences, and also the fact that in some countries the disease has so far occurred only sporadically (Yugoslavia, Czechoslovakia, Romania), veterinary authorities are divided on the best prophylactic measures:

- some countries (Switzerland and Denmark) carry out progressive slaughter of positive animals;

- others (e.g. France and Great Britain) stipulate that AI bulls must be free from infection, because IBR/IPV virus can be excreted in and transmitted by semen. For that purpose, these bulls must have been subjected when young to the serum neutralisation test with negative results and remain negative for all their life;

- a third type of control programme (Federal Republic of Germany) is vaccination of AI bulls.
As far as female cattle are concerned, only Denmark and Switzerland seem to be engaged in a programme of eradication by slaughter. Elsewhere, vaccination is used either freely (Federal Republic of Germany, Great Britain, Netherlands) or restricted and controlled (France).

It is difficult or impossible to put forward a common programme for prophylaxis. The ideal is certainly eradication of the virus from breeding stations, AI centres and pedigree herds. The correct strategy depends on the degree of contamination in the region or country. Recourse to vaccination is justified only in regions of high risk and with a high degree of virus contamination. Males should not be vaccinated unless it is certain that they are not carrying the virus. Only inactivated vaccines should be used. Periodic testing of semen for the absence of virus ensures its safety.

The problems of prophylaxis for breeding stock are quite different from schemes suitable for the rearing of beef cattle, where the essential requirement is to avoid economic loss until the animals are slaughtered. In addition, it is important in breeding stock to monitor the course of infection by means of the neutralisation test, a possibility which is precluded when vaccination is practised.

**CHLAMYDIAL INFECTIONS**

Although less frequent in cattle than in sheep, *Chlamydia* infections can cause reproductive disorders in cows and bulls, and incidents have been recorded in Europe since the 1970's (Polak *et al.*, 1984).

Recognised for some time as the cause of epidemic abortion in cows in the USA, *Chlamydia psittaci* is associated in Europe with sporadic abortion (at 3 to 7 months) due to necrotic placentitis and a direct effect on the fetus (producing liver lesions). Jaskowski (1973) and Jahn *et al.* (1972) also describe infertility with vaginitis and endometritis.

In the bull the infection results in balanoposthitis, inflammation of the vesicular gland (seminal vesiculitis) and above all orchitis. Several outbreaks have been reported in Europe (Poland, Czechoslovakia) and investigated by Jaskowski *et al.* (1980) and Rob and Rozinek (1976). *Chlamydia* can contaminate the semen of infected bulls, but in an irregular, intermittent and feeble way. Guérin (1983) succeeded in isolating *C. psittaci* only once from 8 ejaculates of one bull, and once from 12 ejaculates of another; the semen of two other infected bulls was constantly negative. At the same time all cultures performed with 134 doses of frozen semen from 10 bulls gave negative results. This demonstrates the difficulty of isolating *Chlamydia* in low concentration from semen. The presence of proteases means that the semen has to be diluted 1:10, further decreasing the already low concentration of the organism. It is also difficult to recover the organism from diluted, frozen semen because of the presence of antibiotics in the diluent, which are toxic for *Chlamydia* in the concentrations normally used.

There is little information on transmission from male to male, and the conditions required for disorders to occur, although the oral route of infection seems to be the commonest, supplemented by indirect transmission through tick bites. Experimental transmission of the disease is difficult, even when strains of *Chlamydia* from clinically infected animals are used. *Chlamydia* may occur in the semen of clinically healthy bulls.
Insemination of a cow with contaminated semen may lead to disorders (infertility with vaginitis and endometritis), but it has not yet proved possible to establish the amount of an infective dose, or a suitably receptive substrate.

Diagnosis is relatively easy in the case of abortion, by means of bacteriological examination of the liver of the aborted fetus (stained smears and culture in embryonated eggs). Isolation from bull semen is difficult. Spencer et al. (1983) described a transport medium to aid the isolation of *Chlamydia* from samples, capable of preserving the organisms for 30 days at room temperature and 34 days at +5°C.

The serological technique generally used is complement fixation, but it is difficult to interpret the titres obtained, since they are often low and of limited persistence (a few weeks). The test may also detect antibodies to *Chlamydia intestinalis* (a non-pathogenic saprophyte) in addition to *C. psittaci*. Complement fixation enables a herd diagnosis to be made, but cannot be used to diagnose individual cases.

Recommended treatment is by antibiotics (tetracycline or chloramphenicol), but it is uncertain if they are sufficiently effective and safe to use on bulls (possible adverse effect on semen production). In cows, daily injection of 3-5 g of tetracycline for 4-5 days prevented abortion in pregnant cows in contact with a cow which had aborted as a result of *Chlamydia* infection (Vanderlasche, 1982).

Although the transmission of *Chlamydia* from sheep to cattle has not been proved, it would be wise to separate rams (and ewes) from bulls at an AI centre.

**MYCOPLASMOSES**

The numerous mycoplasmas involved in diseases of cattle create a complex situation with regard to lesions and the causal agent. Species commonly associated with genital tract infection in Europe are *Mycoplasma bovigenitalium, Mycoplasma bovis, Acholeplasma laidlawii* and *Ureaplasma*. Bell (1984) reported the localisation of *M. canadense* in the prepuce and urethra in a herd of bulls.

The aetiological role of mycoplasmas is more often assumed than proved by the relative frequency of occurrence of the organisms in samples from placentas, vaginal discharge, prepuce, urethra and semen. It is difficult to assess the economic impact of mycoplasmas, for they are often found in apparently healthy animals. Jurmanova et al. (1979) found *Ureaplasma* in 80% of 3,000 doses of semen, and *Mycoplasma* in 40%. Rae (1982) found *Mycoplasma* in 39 (71%) of 55 samples of undiluted semen from 10 bulls. Nine samples yielded a combined culture of *M. bovigenitalium* and *Ureaplasma*, and two a combined culture of *M. bovigenitalium* and *M. canadense*. However, the author was unable to find any link between these isolations and the fertility of the breeding stock. Ruhnke and Doig (1978) found a high frequency of *Ureaplasma* and *M. bovigenitalium* in cases of acute vulvitis with hyperaemia and tissue granulation.

Cases of vesicular gland inflammation (seminal vesiculitis) were reported by Erno and Blom (1967) and Al Aubaidi et al. (1972), and this condition has been reproduced experimentally by inoculating the associated mycoplasmas into the gland (Parez et al., 1977). The infection reduced the motility of spermatozoa.

Mycoplasmas can survive in frozen semen.
The antibiotics usually added to diluents (penicillin and streptomycin) are generally ineffective in controlling mycoplasma contamination of semen. Truscott (1983) examined the activity of various antibiotics and found that minocycline was effective against *Ureaplasma*, while lincomycin (0.3 mg/ml) plus spectinomycin (0.6 mg/ml) was effective against *Mycoplasma*. However, the practical application of these results was hampered by the time of contact required (15 min at 35°C), and the influence of the diluent (monocycline being fully effective only in milk diluent).

Neither serological testing of breeding stock nor examining semen for mycoplasmas provide a basis for prophylactic measures, because of uncertainty about the significance of the results.

**OTHER DISEASES**

**Bovine viral diarrhoea/Mucosal disease.**

The widespread virus of bovine viral diarrhoea (BVD) mainly infects young cattle, 8-18 months old, but it can be pathogenic for the pregnant cow, producing abortion in the 3rd or 4th month. It may also be responsible for early embryonic mortality. If the fetus should become infected at a later stage, parturition will take place at full term.

The virus can be excreted in the semen of an infected bull without any abnormality of the genital organs or semen. Whitmore *et al.* (1978) infected nine bulls 2-4 years old with BVD virus by nasal, oral and intramuscular routes, and recovered the virus from the semen after 2, 4 and 10 days from 3 serologically positive bulls and one serologically negative bull. There were no lesions of the genital system and no alteration in semen quality. Insemination with virus-contaminated semen appears to reduce the chances of fertilisation (Archbald *et al.*, 1977). However, McClurkin (1977) found that fertilisation occurred normally in cows which were serologically positive before insemination.

Serological testing of breeding bulls cannot be used in any prophylactic programme for AI centres. It is difficult to isolate the virus in cell culture.

**Listeriosis.**

Known to occur in cattle in a genital form, listeriosis can be responsible for sporadic abortions.

The causal agent, *Listeria monocytogenes*, is very resistant and is ubiquitous. The occurrence of listeriosis in cattle seems to be related to the feeding of silage of poor quality (pH greater than 5).

Abortion results from necrotic placentitis, accompanied by liver and spleen lesions, and abortion occurs between 6 and 8 months. There is constant and persistent excretion of *Listeria* after abortion, whether endometritis is present or not. In France, listeriosis accounts for 1-2% of non-brucellar abortions (Goyon, 1980).

Treatment is usually futile, even with antibiotics.

Prophylaxis entails the careful preparation and storage of silage.

No one has been able to demonstrate *Listeria* in semen.
Leptospirosis.

The pathogenicity and virulence of the various serotypes of *Leptospira interrogans* varies considerably from country to country. According to Uwland, serotype *pomona* is absent from the Netherlands, but is the commonest serotype in the USA. The commonest serotypes in Europe are *grippotyphosa* and *canicola*.

Excretion of leptospires by an infected animal takes place from the bladder (urine), mammary gland (milk) and genital system (semen). An animal is usually infected by ingesting feed contaminated by the urine of other infected cattle, or by the excreta of rodents.

There seems to be little risk of a cow becoming infected by leptospires in semen, because of the small numbers present, and the presence of antibiotics active against leptospires (e.g. streptomycin) in the diluent.

Mycotic abortion.

This may account for 10% of sporadic abortions (Vanderlasche, 1982), and is caused mainly by *Aspergillus fumigatus* present in mouldy forage and hay. Abortion, as a result of placental infection, takes place in the 7th or 8th month.

Various types of fungi, including moulds, have been detected in semen, but it is difficult to determine if this is due to pollution or contamination.

Exclusion of mouldy forage from the feed should prevent this form of abortion.

Salmonellosis.

Although *Salmonella dublin* and *S. typhimurium* can cause sporadic abortions in cows, there is no evidence that the bacteria are transmitted by semen.

Bluetongue in cattle.

Bluetongue virus may contaminate bull semen and be transmitted to cows by the uterine route. The virus is common in North America, but so far absent from Europe.

Akabane disease.

First reported from Japan, next Australia and then Israel, this viral disease has not yet been reported in Europe. It is characterised by arthrogryposis, hydranencephaly and muscular atrophy, and in 1979/80 was responsible for epidemics of abortion (some 50,000 cases), premature calving and the congenital arthrogryposis syndrome (Inaba, 1980). The virus may be present in semen.

The disease is detected by the presence of neutralising antibody in blood serum.

Enzootic bovine leukosis.

It is generally agreed that the oncovirus of leukosis is not transmitted by semen, despite the experimental transmission to sheep by means of large doses of semen and accessory gland secretion (2-10 ml) from an infected bull (Lucas et al., 1980). However, it should be noted that semen contamination by blood (and therefore by polynuclear cells) may constitute a risk if virus is present in the polynuclear cells, because only a very small amount is required for transmission.
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USUALLY NON-PATHOGENIC MICRO-ORGANISMS WHICH MAY BE PRESENT IN SEMEN

Collection under sterile conditions has shown that semen from the vesicular glands is free from micro-organisms (Nibart, 1976).

Ejaculated semen may become contaminated by non-pathogenic, ubiquitous bacteria which are invariably present in the prepuce and the terminal portion of the urethra. The degree of bacterial contamination depends on the standard of hygiene under which the bulls are kept, and on the hygienic precautions taken during semen collection for AI.

The degree of bacterial contamination reported in various publications varies considerably. A detailed review by Wierzbowski (1981) gives mean counts of 150,000 to 650,000 bacteria per ml of freshly-collected semen, and from a few hundred to 500,000 bacteria per ml of diluted semen; only 4% of ejaculates were free from bacteria. Very different data were presented by Balachov (1984), the mean bacterial count of fresh semen being just 3,094 per ml, with 28% of samples free from bacteria. These low figures may be the consequence of using a specific technique for semen collection, not specified by the author.

The commonest bacteria involved are *Pseudomonas aeruginosa*, *Corynebacterium pyogenes*, beta-haemolytic streptococci, staphylococci, *Escherichia coli* and *Proteus* sp., which also happen to be the bacteria most often involved in infections of the female genital system. However, there is little evidence of their pathogenicity for the female genital system, apart from cases with reported excretion of the pathogen (*E. coli*) in the semen of bulls with genital tract (vesicular gland) infection (Blom *et al.*, 1964; Thal *et al.*, 1959). Neither is there any evidence of a relationship between these potentially pathogenic bacteria and the fertilising ability of semen samples. On the contrary, it has been shown that the presence of 5,000 bacteria (*Pseudomonas aeruginosa*) per ml of semen had no effect on the results of AI (Kondrastow, 1975).

Of course, the objective should be to produce semen containing as few of these bacteria as possible. In the present state of knowledge it is impossible to place a limit on the acceptable degree of contamination, apart from defining a maximum count for inflammatory (polynuclear) cells, which is a more reliable indicator of the state of the male genital system than the bacterial count.

The presence of micro-organisms in semen used for AI raises the problem of their relevance to the female animal inseminated, particularly the risk of post-insemination endometritis caused by non-specific bacteria. The role of these bacteria in endometritis is still controversial, since the same bacteria (*Escherichia coli, Pseudomonas, Staphylococcus, Proteus*, etc.) may be present in the uterus of healthy cows, including cows inseminated with semen from healthy bulls (Dawson, 1975; Easley *et al.*, 1951). A recent study of the irrigation fluid used for collecting embryos from donor cows (which are, of course, fertile) has shown that, excluding contamination during the process of collection, it is very rare to obtain bacteriologically negative samples from the uterus (Mallek, 1984), and that the density of the bacteria was very similar to that of the prepuce and semen of bulls. During oestrus the uterine microbial flora of healthy cows increases as a result of the cervix opening, but local and general defence mechanisms keep the number under control by:
— increasing the number of macrophages (capable of phagocytosis) in the uterine wall;
— the intervention of cellular immunity in this mobilisation and in the humoral immunity mediated by IgG and IgA, resulting in the bacteria being confined to the endometrial surface;
— intervention of agglutinins normally present in the uterine mucosa of cows, active against non-specific bacteria.

It seems probable that metritis or endometritis in the cow caused by non-specific bacteria is initiated by disorders of these defence mechanisms (during high proges-terone levels, nutritional imbalance, etc.), or when the pathogenicity of the bacteria is enhanced by a particular combination, excessive numbers or high pathogenicity of a given strain. The role of such factors has not yet been elucidated, but they provide justification for precautions to limit the bacterial contamination of semen.

The objectives should be:
1. Standardise techniques for evaluating the bacterial content of ejaculates and prepared semen.
2. Define the relationship between fertility and bacteria in semen.
3. Elucidate conditions under which the bacterial flora of the bovine uterus may become pathogenic during oestrus.
4. Formulate hygienic precautions to reduce the bacterial content of semen.

These objectives were placed before an FAO meeting of experts on the control of diseases transmitted by semen and embryo transfer, held in Rome in 1981, and they were published as recommendations. Similar proposals have been made by the International Standardisation Organisation (ISO) concerning international trade. The difficulty in establishing a limit for bacterial content is expressed in the following recommendation: « If an importer stipulated that the semen must be free from micro-organisms which are occasionally pathogenic and/or non-pathogenic, a list of the bacteria and the maximum permissible count should be established by agreement between the two parties » (ISO, Kharkov, 1983).

**MICRO-ORGANISMS TRANSMITTED AS A RESULT OF EMBRYO TRANSFER**

Recent developments in embryo transfer raise the problem of health risks associated with this technique, and has led the authorities to consider the basis for regulations.

Such regulations require information concerning the health status of the parents (donor cow or AI bull), and depend on the state of knowledge concerning the risk of an embryo becoming infected from parents infected with a disease of the genital system.

The considerable research conducted during the past few years has shown that the embryo at the blastocyst stage (intact zona pellucida) is unlikely to become infected before implantation. It cannot carry the causal agents of a generalised or genital disease from a female serologically positive and/or clinically infected with *Campylobacter, Brucella, Trichomonas* and other pathogens. Any risk of carrying
a virus (such as IBR/IPV virus, BVD virus) adsorbed to the zona pellucida could be overcome by suitable treatment with immune serum or trypsin, without adversely affecting the survival of the embryo (Singh, 1984).

It has been demonstrated that embryo transfer is a means of obtaining disease-free offspring from infected parents, and to breed from animals of high genetic value but infected with a genital disease. However, there is still a need for research on interactions between embryos and pathogenic micro-organisms (Hare, 1984).

A series of pertinent recommendations for controlling infectious agents during embryo transfer have been presented by Hare (1983).

HYGIENIC PRECAUTIONS TO OVERCOME DISEASE RISKS IN SEMEN PRODUCTION

It seems to us essential to include in the hygiene of semen production the health hazards associated with each step of the process, rather than the narrow aspect of just semen collection, important though this is.

The importance of optimum fertility coupled with rigorous health safeguards is borne out by the enormous extent to which AI is used in cattle, often completely replacing natural service; in Europe some 65 million cows calve each year as a result of AI, and there is a large international trade in semen, about a million doses being exchanged to and from Europe each year. In view of this great importance, the European Commission has recently proposed to member countries a directive concerning the safeguarding of animal health in the exchange of bull and boar semen within the Community, and its importation from countries outside the EEC (Economic and Social Council, 1984).

It is obvious that safeguarding the health of semen depends on:
— the disease-free status of the breeding stock;
— the conditions of isolation under which the breeding stock is kept;
— the hygienic conditions of semen collection;
— hygienic precautions during the preparation of semen doses.

The need for good hygiene in the act of insemination by the inseminator is also of considerable importance for the recipient cow and the herd as a whole. The farmer is entitled to expect a certain standard regarding the wearing of suitable clothing, disinfection between each herd (facilitated by using plastic overtrousers and overboots), and disposable gloves. Such precautions help to avoid the major contamination which would otherwise occur.

Freedom of the bulls from major generalised and genital diseases is usually guaranteed by negative results to biological, allergic or serological tests. The value of such a guarantee depends on the precision with which the various procedures (inoculation, sample collection, laboratory testing) are carried out, and the criteria used for evaluating the results and their reliability.

Difficulties arise when "aberrant" reactions occur (e.g. to brucellosis and IBR/IPV virus), but more modern, complex and precise tests can overcome such difficulties without putting the genetic stock at risk. The preparation of cultures from semen, under precisely defined conditions, can remove doubts (concerning
FMD and IBR/IPV infection) even if it cannot provide absolute proof. (The only proof provided by culture is a positive isolation of a micro-organism.)

The list of diseases for which bulls are usually tested in most European countries comprises tuberculosis, foot and mouth disease, brucellosis, campylobacteriosis, trichomoniasis, leukosis and coital exanthema (IBR/IPV). There seems to be no trend towards adding other diseases to the list. To justify an addition there would have to be an increased occurrence of clinical cases of a disease, followed by investigation of the frequency of excretion of the pathogen by AI bulls (and not just the frequency of antibody carriers).

The sporadic nature of abortion and/or infertility due to chlamydia, mycoplasmas, leptospires and BVD virus in cows justifies vigilance and surveillance of any clinical case occurring in an AI bull, but no veterinary authority has so far considered it necessary to introduce systematic control measures, not even Czechoslovakia, where chlamydiosis is responsible for chronic disease in bulls (testicular dystrophy and orchitis), and more than 20% of cows which aborted possessed serum antibody (Polak, 1984).

Standardisation of the diagnostic tests best suited to detect these various diseases would avert the present serious ambiguities and would facilitate international comparisons. This would make international trade much easier.

Quarantine measures sufficiently rigorous in duration and in the absence of contact should provide adequate protection, which only ignorance of the laws of contamination on the part of the personnel can invalidate. During quarantine the animals are examined clinically and submitted to the various diagnostic tests before being admitted to an AI centre. A newly-admitted bull is tested at relatively frequent intervals (3, 6 or 12 months according to the disease being tested for).

Female cattle used as teasers should be submitted to the same procedures.

The hygiene of semen collection and the preparation of semen doses governs the extent of contamination with non-pathogenic or occasionally pathogenic microorganisms.

The staff of an AI centre should be instructed in the rules of hygiene and the methods of implementing them.

A combination of measures capable of considerably reducing the number of micro-organisms in semen has been proposed by Ostachko (1983) working at the Kharkov AI Centre (USSR). Others have also proposed similar precautions, and Parez (1983) has regrouped them as follows:

— AI bulls should never be allowed to serve a cow;
— the preputial sac and orifice of semen producers should be examined regularly, and any bull with balanitis and/or acrobustitis should be withdrawn from semen production;
— semen should not be collected from bulls having a pendulous prepuce or eversion of the preputial mucosa (because both conditions favour contamination of the prepuce);
— before semen collection the abdomen and prepuce (from which the hairs have been cut) are washed with soapy water and then dried;
— irrigate the prepuce with 250 ml of sterile saline solution about 15 min before semen collection. While the microbial flora of the prepuce may be reduced temporarily by using solutions of disinfectants or antibiotics, the original degree of contamination will be resumed after 2 weeks. Repeated use of disinfectant solutions may result in colonisation by a resistant or even pathogenic flora, with the complication of balanoposthitis;

— the litter (bedding) should be changed regularly, using the same standard of hygiene as practised in cowsheds to reduce infections among cows;

— semen collection should take place on grass, or on a non-slip surface capable of being easily washed and disinfected; avoid sand and sawdust;

— when a teaser cow is used, its hind parts (which come into contact with the bull's penis) should be disinfected after each collection, or better still, cover the hind parts with a sheet of plastic, renewed for each collection;

— collection should be abandoned if the teaser cow defecates before or during the collection;

— the operator should wear protective clothing which can be cleaned (plastic or rubber) and a disposable glove should be used on the hand which grasps the penis;

— collection is made after only one penetration of the penis into the artificial vagina;

— the artificial vagina should be disinfected before each use;

— preference should be given to sterile, disposable materials rather than rubber;

— immediately after collection, the semen should be protected from contact with the outside air;

— the laboratory for semen preparation should have its own entrance, away from the main entrance to the AI centre;

— diluent should be prepared from fresh, sterile products and with sterile equipment. The diluent is a biological medium capable of supporting bacterial growth;

— packing materials for doses of semen should be sterile, and packing should take place out of contact with the surrounding air;

— doses should be for a single animal, hermetically sealed.

Antibiotics have been added to diluents since the 1940's, and at that time they were valuable for destroying Campylobacter in semen. This bacterium has now become a rarity, and the freezing of semen is now widespread, yet streptomycin and benzylpenicillin are still used. The question should be asked whether the duration of contact at temperatures above 5°C (30 min) or at freezing point (3 h) are adequate to permit these antibiotics to act. Is the range of activity of these antibiotics still suitable for the bacteria they are meant to destroy (Chlamydia, Mycoplasma, Listeria, Leptospora, Salmonella and the ubiquitous bacteria)? Truscott et al. (1983) demonstrated the effectiveness of lincomycin, spectinomycin and minocyclin (alone or in combination) against Mycoplasma and Ureaplasma, and they established the limits of activity of these antibiotics. There is a need for further research on the choice of available antibiotics. Another potential line of research is the possibility of immunological intervention, using either specific antibodies (and the initial results of our own experiments have been promising) or active fractions of serum (immunoglobulins) for adding to semen diluents.
Nevertheless, «the use of antibiotics in semen should not be allowed to serve as an alternative to declaring a group of bulls free from disease» (Bell, 1984).

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**

REFERENCES

(see p. 65)