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MICROBIOLOGICAL INVESTIGATION ON FROZEN RABBIT SEMEN

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INTRODUCTION

The interest in artificial insemination (A.I.) among breeders in Italy has increased rapidly over the last few years, following experimental progress in this field (Costantini 1986, Facchin et al. 1988, Brivio et al. 1989).
Even though the introduction of this technique has resulted in an overall improvement in the management of the rabbitries, there have been some negative aspects regarding fertility and a considerable increase in abortions and metropathies in the does which had been subjected to A.I. over a period of time was noticed during these studies.
It was suggested that the semen was responsible for the transmission of pathogenic or potentially pathogenic bacteria. To evaluate this possibility, we carried out a series of tests on the ejaculate of some male examples from rabbitries, routinely using this technique.
It is a well-known fact that the semen of many mammals is not sterile. Riley and Masters (1956) found bacterial contamination in 68% of the 242 samples of human semen examined; Zemjanis (1970) believes it is difficult to obtain sterile bovine semen and Roberts (1979) encountered the same difficulty with stallions.
An extensive research project by Danowski (1989) on the bacterial content of swine semen showed the presence of 13 genera of Gram-negative bacteria and 4 genera of Gram-positive ones. The average was 1,3 x 10 organisms/ml.
Mercier and Rideaud (1991) have recently investigated the same problem in the rabbit. They obtained values which varied from 1020 to 60800 organisms/ml, depending on the conditions of the rabbitry the semen came from.

MATERIALS AND METHODS

Two commercial rabbitries which have used A.I. as the only breeding procedure for many years, were considered for the investigation. Rabbitry 1 consisted of 2000 does. Rabbitry 2 consisted of 700 does.
The hygienic standard of rabbitry 1 was good while rabbitry 2 had a slightly lower standard. In rabbitry 2, the males are kept in a separate room.
Both rabbitries used the same instrumentation for collection and insemination: artifical vagina IMV France, latex sheath, glass

catheters and glass tubes.
During week 1, the same latex sheath was used to collect the ejaculate from several males (Method A). The following week the sheath was changed at every ejaculation (Method B). The two methods were repeated alternately until the end of the test which lasted a total of 6 weeks. Each week, the same selected males, 5 from rabbitry 1 and 5 from rabbitry 2, were required to provide two samples collected with a time lapse of about 20 minutes.
The two ejaculates of the same male were treated in the way they were normally prepared for commercial use.
Following macroscopic evaluation (for identification of volume, colour and presence foreign bodies), the semen was diluted 1:1 and examined microscopically to evaluate motility, vitality, and density of the nemasperms. On the basis of the evaluations obtained, the ejaculate was further diluted, generally in the ratio 1:10.
The buffer (DILAP 2000, IMV France) was treated with Penicillin, Streptomycin and Gentamycin antibiotics by the manufacturer.
Each diluted sample was stored in a single tube at 4° C until used (about 15 minutes later).
On average, about 15 doses of 0.5 ml were sampled from each tube, using an equal number of sterile glass catheters, which are, in turn, used to inseminate the female rabbits. The first and last doses, for a total of 89 samples were collected in medium-sized paillettes and immediately stored under liquid nitrogen to avoid any microbial alteration prior to bacterial examination. Each sample was subjected to qualitative and quantitative bacteriological analysis.
The count of the microrganisms was done by a modified version of the micromethod devised by Zavanella et al (1983). This method was used for the determination of the total content of mesophyllic bacteria, E.coli and coliforms, staphylococci, and sulphate - reducing clostridia (Tab.I).
For the qualitative analysis, plates were seeded with Blood Agar, Serum Agar, MacConkey Agar and identified by the API SYSTEM micromethod (API 20 E, API 20 NE, API STAPH, API 20 STREP). The negative lactose colonies isolated from the MacConkey Agar were assayed with an agglutinant, anti-Bordetella bronchiseptica serum (Istituto Zooprofilattico Sperimentale, BS, Italy).

The 780 inseminated females (469 from rabbitry 1, 311 from rabbitry 2) were followed from a health aspect as well as from the aspect of fertility and prolificacy (Fig.2). In this trial, 2,079 live births were recorded in rabbitry 1 and 1,109 in rabbitry 2.

The statistical analysis of the data was elaborated using Student's test. A correlation was carried out between the number of bacteria and reproductive efficiency.

RESULTS

In absolute terms, the microbial count was higher in the bucks from rabbitry 1, when subjected to sampling with a repeatedly used sheath (Method A)(Fig.1). With the second collection in particular, an overall increase in the microbial contamination was

found in all the males, both with the first and the last dose. These increased values were due to the extensive presence of enterobacteria with prevalence of Proteus spp. Only 5 out of the total of 89 samples examined resulted sterile. 4 of these originated from rabbitry 2 and only one sample from rabbitry 1 (Tab.II). The average bacterial count varied considerably, not only between the two rabbitries and between the two types of collection (Method A or B) but also within the same male population itself and individually too.

In fact, the organisms in samples from rabbitry 1 was, on average, 5 times higher than the count from rabbitry 2. This confirms the good practice, from a hygiene-health aspect, to keep the males in a separate room. However, despite these observations, the differences we found among the average bacterial count from the two rabbitries were not statistically significant, probably because of the very high SD found.

Even the method of collection produced interesting results from a bacteriological point of view. For example, the bacterial count in rabbitry 1 decreased from 285,000 organisms/ml found with Method A to 16,000 organisms/ml with Method B (last dose). In rabbitry 2, similar readings were observed (49,000 organisms/ml against 8.000 organisms/ml). Analogous differences were recorded between the first and last dose, irrespective of the collection method.

A qualitative analysis was done by the isolation of Pasteurella spp. and of Bordetella bronchiseptica, as well as showing the microbial flora normally present in such a low quantity as to not be revealed by quantitative analysis (< 50 organisms/ml).

As can be seen in Table III, 7 genera of Gram-positive and 4 genera of Gram-negative bacteria have been identified. In the 52 samples examined, Pasteurella pneumotropica was only found once, while Staphylococcus aureus was found in 28, E.coli in 22, and Cl.perfringens in 12 of the samples.

The other species identified can be considered scarsely or non-pathogenic. However, in the females we inseminated, the resulting fertility and the percentage prolificacy was comparable to the average of the rabbitry (Fig.2). This is despite the negative correlation between the live births and the more highly contaminated semen originating from rabbitry 1. This correlation was not statistically significant, in any case.

DISCUSSION

The bacteriological results we found were extremely variable due to several factors: the type of rabbitry, the timing of the sampling and the method of collection. Paradoxically, the organisms/ml were much higher in the semen from males originating from rabbitry 1, which appeared to have a better standard of hygiene. As a matter of fact, the isolated room, used in rabbitry 2 to house males only, appeared to be advantageous in avoiding this

type of contamination. This is despite the more unhygienic appearance of this rabbitry.

Only the high standard deviation found between the individual bacteriological analysis impeded the illustration of the statistical significance of the readings from the two rabbitries, but the difference is clearly evident (Tab.II).

The substitution of the latex sheath after every collection was shown to be efficient in reducing the organisms 50 times. However, using this system, a large quantity of staphylococci remains, perhaps as a result of contamination by the operator and certainly due to the increased manipulation of the equipment.

Working in field conditions, the considerable differences registered between the first and last dose of each sample was expected, given the inevitable and progressive contamination of the multidose tubes. The different bacterial count found among the various males during the 6 test-weeks would appear to be a problem more directly linked to accidental contamination of the ejaculate at the moment of collection than to primary microbial presence on the male genital organs.

In Fig.1, it can be seen that the higher values are never registered by the same producer, but they vary each time. No external lesion of the genitals was observed and the reproductive performances had been maintained, even when the bacterial flora reached extremely high levels (2.000.000 organisms/ml) in the semen.

We often found such high values in semen samples from other sources which we had previously tested (non-published data).

Even though our data differ greatly from the average results from other studies on bovine (Kendrick et al. 1975), swine (Danowski, 1989) and rabbit semen itself (Mercier and Rideaud, 1991), we believe that the biological quality of the semen under examination has not been endangered. The confirmation of this is the good reproductive performances obtained by the inseminated females (Fig.2).

Under our field conditions, with the exception of 5 cases of the 89, bacteria were found in diluted rabbit semen, even after an addition of antibiotics to the buffer.

Among the factors which negatively influence the bacteriological quality of the semen, we have identified:

a) the density of the rabbit population, as opposed to the apparent hygienic conditions of the rabbitry itself;

b) the use of a single sheath for several collections;

c) the use of multidose tubes;

Taking advantage of the experience obtained in the bovine field,

Kenrick et al (1975), regarding the rabbit we suggest the following points:

1) keep a room exclusively for the male donors. It must have easily washable walls and floor in order to remove the daily excreta and to keep the hutches clean at all times;

2) It is necessary to sterilize used sperm recepients, the artificial vaginas and the catheters used for A.I. This applies only when it is not possible to use disposable instrumentation.

3) during the insemination of the female, avoid touching the perineum with the catheter. It is often smeared with faeces and provides a risk of introducing pathogenic micro-organisms into the vagina.

We believe that the future of A.I. in rabbit breeding depends on the observance of extreme professionalism during all the operating phases, starting with the healthy selection of the breeders.

In the light of the results of our investigation, we can assume that the abortions and metropathies observed in the rabbitries that routinely use A.I., cannot be attributed to infected semen, but may be due to more serious diseases (Pasteurellosis, Chlamydiosis, Myxomatosis etc.).

We agree with Mercier and Rideaud (1991) that, within certain limits, a correlation between the bacterial and the biological quality of the semen does not exist. Nevertheless, it is important to respect the maximum standards of hygiene and to use a buffer, treated with antibiotics, given the potential pathogenicity of many of the isolated species. It is also important to take into account the reproductive efficiency of the inseminated females which has always remained high, despite the presence of the antibiotics in the buffer, contrary to what was feared by Graczyk and Dubiel (1978).

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FIG.1: TOTAL BACTERIAL POPULATION ON DILUTED SEMEN (3 COLLECTIONS FROM 5 SELECTED BUCKS)

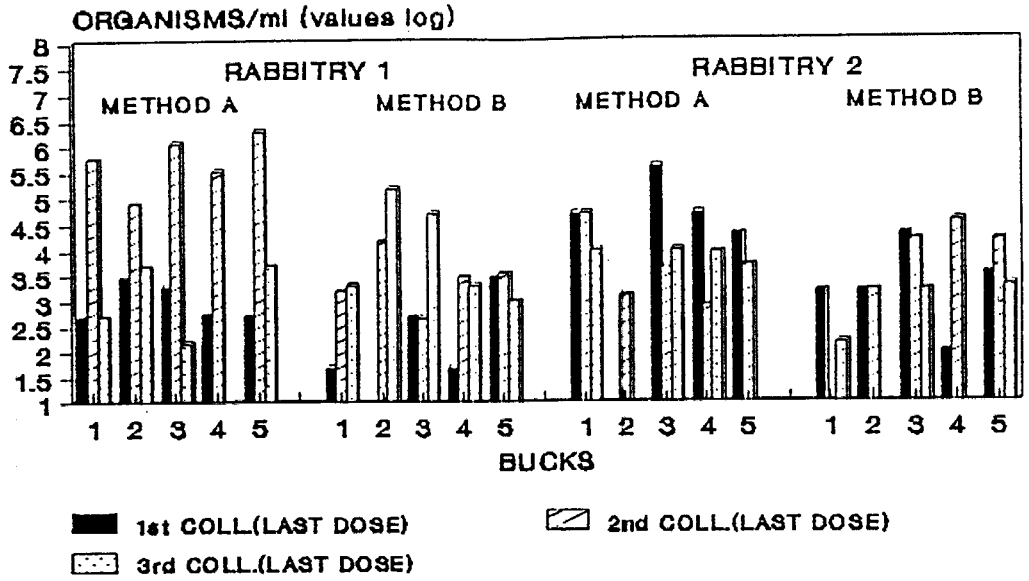
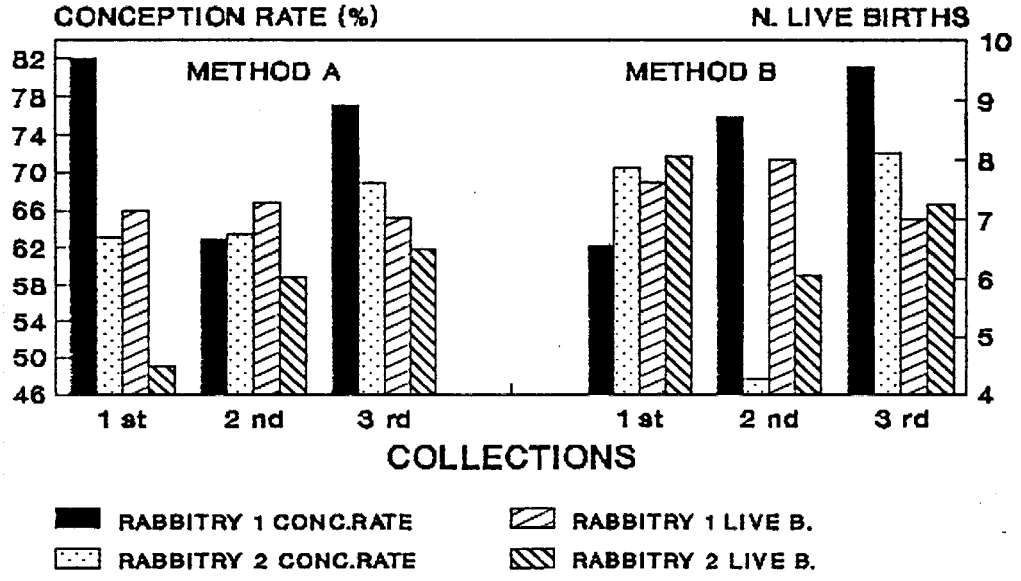


FIG.2: RELATIONSHIP BETWEEN THE CONCEPTION RATE (%AVE) AND THE LITTER SIZE (LIVE BIRTHS, AVE)



COMPARTMENTS	MEDIA	DILUTIONS	MICROBICAL POPULATION	COLONIES TO COUNT
1	Blood agar	ND-1-2-3-4	Mesophilic bacteria	All
2	Mug agar	ND-1-2-3-4	Coliforms, E. coli	Red, fluorescent
3	B.P. agar	ND-1-2-3-4	Staphylococci	Black
4	T.S.C. agar	ND-1-2-3-4	Sulfate reducing bacteria	Black

TAB. I - Media, dilution and reading of the differential tests on dish compartment (Zavanella et al. 1983, mod.)

		Rabbitry 1				Rabbitry 2			
COLLECTION METHOD		A		B		A		B	
DOSE		first	last	first	last	first	last	first	last
N. of SAMPLES		9	14	11	14	13	12	4	12
STERILE		0	0	1	0	2	1	1	0
N. Organisms/ml	AVE.	87800	285000	1600	16500	250	49000	630	9000
	S. D.	164500	577000	1650	39100	250	107000	630	10400
POSITIVE SAMPLES FOR									
E. coli (%)		4 (44)	10 (78)	8 (71)	4 (28)	1 (8)	4 (33)	0 (0)	2 (16)
Staphylococcus (%)		3 (33)	14 (100)	8 (71)	3 (21)	6 (46)	2 (16)	3 (75)	2 (16)
Cl. perfringens (%)		0 (0)	4 (28)	0 (0)	3 (21)	0 (0)	3 (25)	0 (0)	1 (8)
Proteus spp. (%)		3 (33)	7 (50)	1 (9)	0 (0)	1 (8)	2 (16)	0 (0)	7 (58)

TAB II - Bacteriological examinations of diluted semen. S.D.= standard deviation

Nr.	BACTERIA IDENTIFIED	Nr.	BACTERIA IDENTIFIED
28	Staphylococcus aureus	2	Pseudomonas maltophilia
22	Escherichia coli	2	Staphylococcus xilosus 1/2
16	Proteus spp.	2	Streptococcus spp.
12	Clostridium perfringens	1	Aerococcus viridans
5	Proteus vulgaris	1	Enterococcus faecium 2
4	Staphylococcus warneri	1	Enterococcus spp.
4	Streptococcus mutans	1	Gemella haemolysans
4	Enterococcus faecalis	1	Pasteurella pneumotropica
3	Bacillus spp.	1	Staphylococcus epidermidis
2	Enterococcus faecalis 2	1	Staphylococcus haemoliticum

TAB. II - Bacterial identification on 52 samples of rabbit frozen semen (last dose)