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STUDY OF AN ARTIFICIAL VAGINA TO REDUCE THE MICROBIAL CONTAMINATION OF RABBIT SEMEN

DAL BOSCO A.*, SCUOTA S.**, CASTELLINI C.*, CENCI T.**

* Istituto di Zootecnica Generale, Facoltà di Agraria, Università di PERUGIA - Italia
** Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, PERUGIA - Italia

ABSTRACT: Aim of the work was to evaluate and improve the efficiency of an artificial vagina with a short body which permits to collect semen almost directly into the test-tube (with the least contact with the elastic sheath). At the same time, also the test-tube is not in contact with the sheath. Ten adult hybrid bucks were collected during 10 weeks. The experiment consists of 2 successive phases: 1) analysis of microbial flora naturally present in the semen; 2) evaluation of effect of exogenous contamination. Each time, 2 collections were done, using alternatively the prototype and the standard vagina (as a control) at intervals of 20 minutes (maximum 5 times). Throughout the experiment data were collected, concerning operational activities (ejaculation time and reduced sheath temperature from 45 to 35 °C), biological quality of semen (volume, sperms concentration, live sperms) and microbial contamination rate (specific and total flora). The results showed that the time employed to collect the semen was not significantly affected by the different models of artificial vagina used. The microbial contamination of the semen collected with the prototype was lower than the other (phase a, b). The number of collections done using the same vagina did not affect biological characteristics of semen whereas increased progressively the microbiological contamination. A reduction of the contamination at the least 40% can be realized when the prototype is used (phase b).

RESUME : Etude d'un vagin artificiel capable de réduire la contamination microbienne du sperme de lapin.
Le but de ce travail est d'évaluer et d'améliorer l'efficacité d'un vagin artificiel assez court pour permettre la collecte presque directe dans le tube à essai de la semence (avec le moins de contact possible avec la gaine élastique). De plus le tube à essai lui-même n'est pas en contact avec la gaine. Des récoltes ont été effectuées sur 10 mâles adultes hybrides pendant 10 semaines. L'expérience comporte deux phases successives : 1/ analyse de la flore microbienne naturelle contenu dans la semence. 2/ évaluation de l'effet d'une contamination exogène. A chaque prélèvement deux récoltes ont été effectuées, en utilisant alternativement le prototype et le vagin artificiel standard (contrôle) à 20 minutes d'intervalles (5 fois maximum). Pendant l'expérimentation les données concernant les conditions opérationnelles (durée de l'éjaculation, température de la gaine allant de 45 à 35°C) la qualité biologique du sperme (volume, concentration du sperme, vie des spermatozoïdes) et le taux de contamination microbienne (spécifique et flore totale) ont été enregistrées. Les résultats montrent que le temps consacré à la collecte n'est pas significativement différent quelque soit le type de vagin utilisé. La contamination microbienne de la semence collectée avec le vagin prototype est plus faible qu'avec le vagin standard (phase a, b). Le nombre de collectes effectuées avec le même vagin n'affecte pas les caractéristiques biologiques de la semence bien que la contamination microbienne augmente progressivement. Une réduction de la contamination d'au moins 40 % peut être obtenu en utilisant le vagin prototype (phase b).

INTRODUCTION

At present the artificial insemination is widely used in the intensive rabbitries, because of its beneficial influence on management, productivity and breeding (FACCHIN *et al.*, 1991). Hygienical advantages associated with this technique could be very important, since it reduces the risk of transmissible sexual diseases spreading (HARE, 1985).

Many Authors (MERCIER and RIDEAU, 1990; GRILLI *et al.*, 1992) reported that it was very difficult to collect semen with a low microbial contamination rate.

Our previous works (CENCI, 1993, SINKOVICS *et al.*, 1993), indicated that the semen collected from healthy bucks was contaminated mainly by environmental germs and the contribution from endogenous flora of animals was not significant.

The environmental flora found in semen was mainly represented by *Enterobacteriacee*, *Pseudomonas*, *Streptococcus* and *Clostridium* (CASTELLINI *et al.*, 1993). It also appeared that many factors can increase the microbial contamination rate, such as: the contamination of the elastic sheath of the artificial vagina, the orifice of the test-tube used to collect semen, the contamination of buck's perineal zone and collector's hands, especially when an unsterilized vagina is re-used.

To overcome above-mentioned problems, we developed the present prototype of artificial vagina and compared its performance with a model of vagina commonly used in Italian rabbitries.

MATERIALS AND METHODS

The prototype of artificial vagina has two peculiar characteristics: reduced body length, which helps to minimize the contact area between sheath and penis of the buck, and the presence of a thread to fix the test tube without any contact with the sheath (Figure 1).

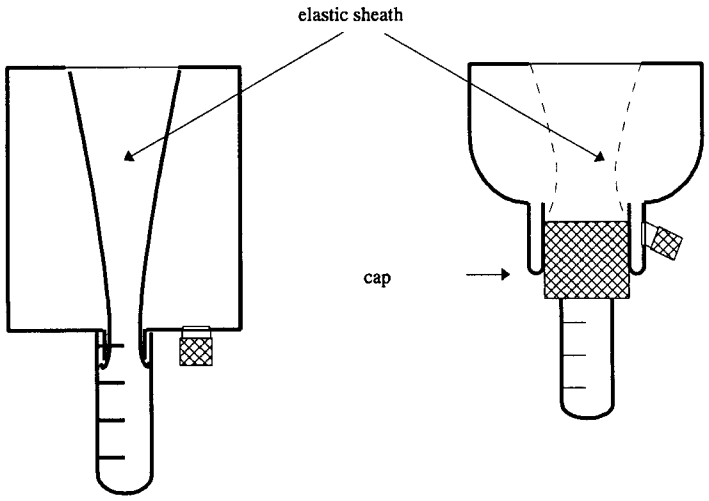
As already mentioned a comparison between this vagina and a standard one has been done.

The characteristics and the dimensions of the two models are presented in Table 1; other parameters (sheath temperature and ejaculation time) were recorded. The first

Table 1: Main characteristics of the artificial vaginas.

	Material of construction	Volume (ml)	Length (mm)	Ø external (mm)	Ø internal (mm)
Vagina standard	Plastic	36	61	52	41
Vagina prototype	Glass	27	38	52	28

Figure 1: Characteristics of the two artificial vaginas



parameter is considered as the sheath temperature at different times (every minute during 10 minutes, starting from 45 °C), while ejaculation time is the period between mating and ejaculation.

During collection the temperature of the elastic sheath was maintained between 40 and 42 °C and vaginas have never been sterilized to reproduce the normal procedures used by breeders; sterilized test-tubes have only initially been used.

Ten adult hybrid bucks, raised in an experimental rabbitry, previously trained for semen collection, have been used.

The experiment included two phases, lasting 6 and 4 weeks respectively.

Phase a : Evaluation of microbial flora naturally present in the semen.

The semen has been collected from each buck twice a week. Each time, at intervals of 20 minutes, two collections were done using alternatively the two different types of vagina for a maximum of 5 times (Figure 2). The main biological traits of semen (volume, concentration, % of live sperms) were evaluated.

From a microbiological point of view, samples have been considered positive when the microbial rate was more than 10 CFU/ml (colony forming unit); concerning the anaerobic germ rate, samples were divided according to the presence or the absence. Undiluted semen was used for bacteriological examination within two hours from collection (stored at room temperature).

For the aerobic germs count, 0.05 ml of sample were plated onto simple Agar, blood Agar, Gassner and McConkey added in glucose 1% and incubated for 24 hours at 37 °C.
After incubation, different types of microorganisms were identified, according to morphological and biochemical characteristics of the colonies.
For the anaerobic germ count, samples were inoculated into Tarozzi and Tioglicollate media and then incubated for 24 hours at 37 °C. From the test-tube in which formation of gas appeared, stained smears and subcultures in Agar SPS (DIFCO) incubated for 48 hours at 37 °C into anaerobic conditions (Gas-Pack System, BBL) were made. For the Fungi isolation the samples were plated onto Sabouraud dextrose Agar containing gentamicine (5 mg/ml), incubated at 25 °C in moist atmosphere for 30 days and examined every day. They were identified by their morphology.

Phase b : Effect of exogenous contamination on the total microbial rate.

To evaluate vaginas in critical operative conditions, an exogenous contamination was carried out spraying 2 ml of *Sarcina lutea* (turbidity equal to 7^{th} degree of the McFarland nephelometer standard) on the perineal area of the bucks immediately before semen collection.

Total microbial count has been done according to TIECCO (1984).

Statistical analyses for continuos variables were done using the following linear model (SAS/STAT, 1990, PROC GLM):

$$y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \varepsilon_{ijkl}$$

where :

y_{ijkl} = experimental observation
μ = general mean
α_i = fixed effect of artificial vagina (j = 1..2)
β_j = fixed effect of collection order (i = 1..5)
$(\alpha\beta)_{ij}$ = fixed effect of the interaction
γ_k = fixed effect of buck (k = 1...10)
ε_{ijkl} = residual experimental error

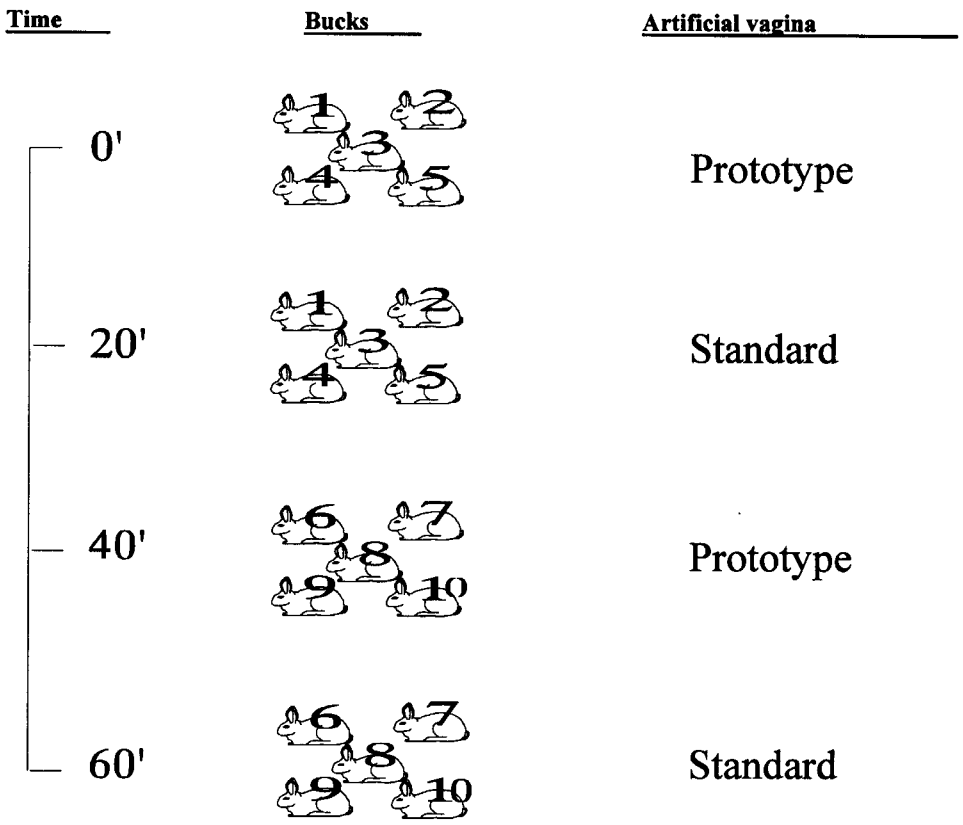
The effects of the buck and of collection order, when not significant, were not reported in the tables.

Categorical data (presence/absence) were analyzed with the Procedure FREQ (CHISQ option).

RESULTS AND DISCUSSION

The two different models of vagina did not significantly affect the time to collect semen (Table 2). The sheath cooling

Figure 2: Protocol of research



In the following week an inverse order (Standard, Prototype) has been used.

of the prototype was quicker than the one of the standard vagina, allowing 1 or 2 collections less (Figure 3).

The volume of semen obtained using the control vagina was slightly higher, probably because of a better contact between the penis and the elastic sheath. The model of vagina did not affect the qualitative characteristics of semen (Table 2).

From the microbiological point of view, in the first phase of the experiment, the microorganisms listed in Table 3 were identified. They represent the most common flora found in the rabbit semen (MERCIER P. and RIDEAU P., 1990, 1992). Results also showed that microbial contamination generally was significantly lower in semen samples collected with the prototype vagina than the control one.

Microbial contamination was also lower in semen samples collected using prototype vagina after the spraying of a *Sarcina lutea* solution (**phase b**), except in the second collection (Table 4).

The order of semen collection increased significantly the bacterial contamination rate. These findings agree with those of CASTELLINI *et al.* (1993).

Semen microbiological contamination could be reduced by 40% using the prototype vagina.

Table 2: Biological parameters of semen.

	Time of collection (sec)	Seminal volume (ml)	Concentration (n x 10^6)	Live sperms (% live /total)
Vagina standard	36	0.85	450	77
Vagina prototype	41	0.78	470	76
SED	15	0.39	110	21

n = 120.

CONCLUSION

According to the results of the present work, we can conclude that it is possible to reduce microbial contamination using a prototype vagina to collect the rabbit semen. When pathogenic or potentially pathogenic microorganisms are present, the reduction of contamination is very important in breeding of rabbits since they can be transmitted.

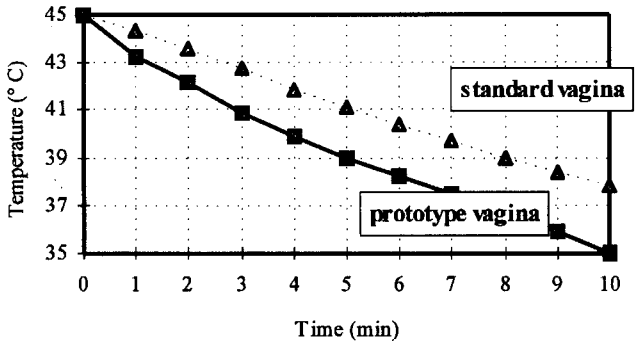
The use of unsterilized artificial vagina for semen collection, as normally occurs in practice, increased microbial contamination; however it is possible to minimize the

Table 3: Level of bacterial contamination in rabbit semen collected with two artificial vaginas.

Micro organism (%)		Vagina standard	Vagina prototype	P	X^2
Streptococcus	<10 CFU	51	83	**	23.2
	>10 CFU	49	17		
Staphilococcus	<10 CFU	56	88	**	25.4
	>10 CFU	44	12		
E. coli	<10 CFU	88	100	*	10.7
	>10 CFU	12	-		
Bacillus	<10 CFU	100	100	n.s.	-
	>10 CFU	-	-		
Anaerobic	A	76	86	n.s.	3.2
	P	24	14		
Fungi	<10 CFU	88	100	*	12.2
	>10 CFU	12	-		

CFU = Colony Forming Units; A = Absence; P = Presence. n = 120; *: P<0.05; **: P<0.01

Figure 3: Sheath temperature of the two artificial vaginas.



contamination using the prototype vagina even under the above mentioned conditions.

The proposed prototype vagina, even if unsterilized and under unfavorable conditions, such as the microbial contamination simulated in this study, permits to collect semen with a low microbial contamination.

Although the present model has some problems, the obtainable advantages suggest that this model can be developed to obtain a more efficient and resistant model (e.g: using Plexiglas instead of glass).

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Table 4: Microbial parameters of semen collected after dirtying with *Sarcina lutea.*

Order of collection	1°	2°	3°	4°	5°	General mean	SED
Total contamination (CFU/ml)							
Vagina standard	11,173 B	31,440	37,446 B	78,764 B	127,207 B	54,827 B	69,500
Vagina prototype	3,609 A	7,827	3,470 A	22,754 A	25,060 A	12,553 A	

n = 80; A..B: P ≤ 0.01.