tttThe Effect of Human, Horse and Fetal Bovine Sera on the Growth of *Trichomonas vaginalis*

TRICHOMONAS VAGINALIS ÜREMESİNE İNSAN, AT VE FETAL BOVİN SERUMUNUN ETKİSİ

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SUMMARY

Objective: It was detected the effect of different sera on the growth of *Trichomonas vaginalis*.

Material and method: Isolates were cultivated in three different modified Diamond media containing human, horse and fetal bovine sera (FBS). The growth rates of the parasite in all three media were investigated after 24, 72, 120, 168 and 198 h.

Results: The growth rates of the clinical isolate 1 were significantly higher than the FBS and horse serum after 24, 72 and 120 h ($p < 0.05$).

Conclusions: This has led us to believe that sera from different living beings used in the *T. vaginalis* culture could yield varying results in different clinical isolates.

Key words: *T. vaginalis*, culture, sera

ÖZET

Amaç: Farklı serumların *in vitro* koşullarda *Trichomonas vaginalis* üremesi üzerine etkisi araştırılması.

Gereç ve yöntem: *T. vaginalis* izolatları insan, at ve fetal bovin serumu (FBS) içeren 3 ayrı modifiye Diamond besiyerine ekildi. Her 3 ortamda parazitin 24., 72., 120., 168. ve 198. saatlerdeki üreme hızı araştırıldı.

Bulgular: Klinik izolat 1'in 24., 72. ve 120. saatlerde üreme hızları, FBS ve at serumuna göre anlamlı düzeyde yüksek bulundu ($p < 0.05$).

Sonuç: *T.vaginalis* kültüründe kullanılan farklı canlılara ait serumların farklı klinik izolatlarda değişken sonuçlar verebileceği kanısına varıldı.

Anahtar sözcükler: *T. vaginalis*, kültür, serum

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Despite the fact that trichomoniasis is not a life threatening disease, it constitutes a serious health problem among women as a sexually transmitted disease (1-3). Because 10-50 % of the cases follow an asymptomatic course, it is not possible to establish a diagnosis based on the history and the clinical findings only (4). As a result of the fact that wet preparation examination of vaginal fluid has proved inefficient in determining all the cases, *in vitro* culture is accepted as the most sensitive method of diagnosis (5-7). The medium which was developed by Diamond and later used in the diagnosis of other protozoa is often used for this purpose (8). The media used in the diagnosis of *Trichomonas vaginalis* also help to throw light on the physiology and metabolism of the parasite (9-13).

Many researchers have reported that the mammalian sera, which is one the main substances found in the *in vitro* culture of protozoa is essential in the provision of lipid, fatty acids and amino acids for the

growth of *Entamoeba histolytica, Giardia intestinalis and T. vaginalis* (14-16). The sheep serum which was added to the initially identified Diamond medium was later replaced by horse sera.

In this study, our aim is to investigate the growth rate and life span of the on two different clinical isolates of *T. vaginalis* after preparing the Diamond medium, with of *T. vaginalis*, with three different sera, namely human, horse and fetal bovine sera (FBS).

MATERIAL AND METHOD

Clinical Isolate and Culture: *T. vaginalis* isolates were obtained from the vaginal secretions of two female patients. After the modified Diamond medium was prepared, it was passed into two tubes in such a way that each tube contained 3ml of the medium, and kept at +4 °C before use (17). In this study, 3 groups were formed with the addition of different sera samples for the two different isolates. For each group, the media in the three tubes were cultivated. 20 % horse sera (Sigma Chemical Co., St Louis, Missouri), FBS (Sigma Chemical Co., St Louis, Missouri) and inactivate human sera obtained from healthy individuals were used separately during the study.

Counting procedure: From the two isolates, cultivation was made into 10-20 ml screw -capped tubes containing modified Diamond medium with a proportion of 10^5 organisms per ml. The tubes of media were inoculated at 37 °C. After 24, 72, 120, 168 and 192 h, motile parasites present in the samples taken from the tubes by means of a pasteur pipette were counted at a haemocytometer and the growth rate of the parasites observed in the isolates prepared with each sera. Prior to counting, in order to obtain a homogenous dispersion of the parasites in the tube, it was vortexed mechanically for 5-10 seconds.

Statistics: One- way Anova (Post hoc; Benforrini) was used for the differences among the groups, and Mann-Whitney U test for the groups demonstrating significant differences.

FINDINGS

The growth rates of the two *T. vaginalis* isolates determined in the modified Diamond medium prepared with all three sera after 24, 72, 168 and 192 h are shown in Table I and Table II. When the growth rates of the both clinical isolates in the modified Diamond media with human, horse and fetal bovine sera were compared and, significant differences were observed after 24, 72 and 120 h ($p < 0.005$, One –Way Anova, Post hoc, Benforrini). No significant difference was found after 168 and 192 h (Figure 1).

Table I. The average growth rates of the clinical isolate 1 in the modified Diamond media with, human, horse and fetal bovine sera

	Number of Cells (X10.000)		
Time(hours)	Horse	Human	FBS
24	6	10	2
72	122	380	140
120	8	67	0
168	0	1	0
192	0	0	0

Table II. The average growth rates of the clinical isolate 2 in the modified Diamond medium with, human, horse and fetal bovine sera

	Number of Cells (X10.000)		
Time(hours)	Horse	Human	FBS
24	8	8	26
72	102	92	118
120	0	0	0
168	0	0	0
192	0	0	0

Findings Related to Clinical Isolate 1: After 24, 72, 120 and 168 h, the growth rates of the clinical isolate 1 in the medium with human sera were found to be significantly higher than those in the media with horse and fetal bovine sera ($p< 0.05$, $p< 0.05$, respectively).

After 24 and 120 h, the effect of the horse sera on the growth rates was greater than that of the FBS ($p< 0.005$), whereas the FBS was more effective than the horse sera after 72 h ($p< 0.005$). (In the comparison of the sera, Mann-Whitney U test was used as a statistical analysis) (Table I, Figure 1).

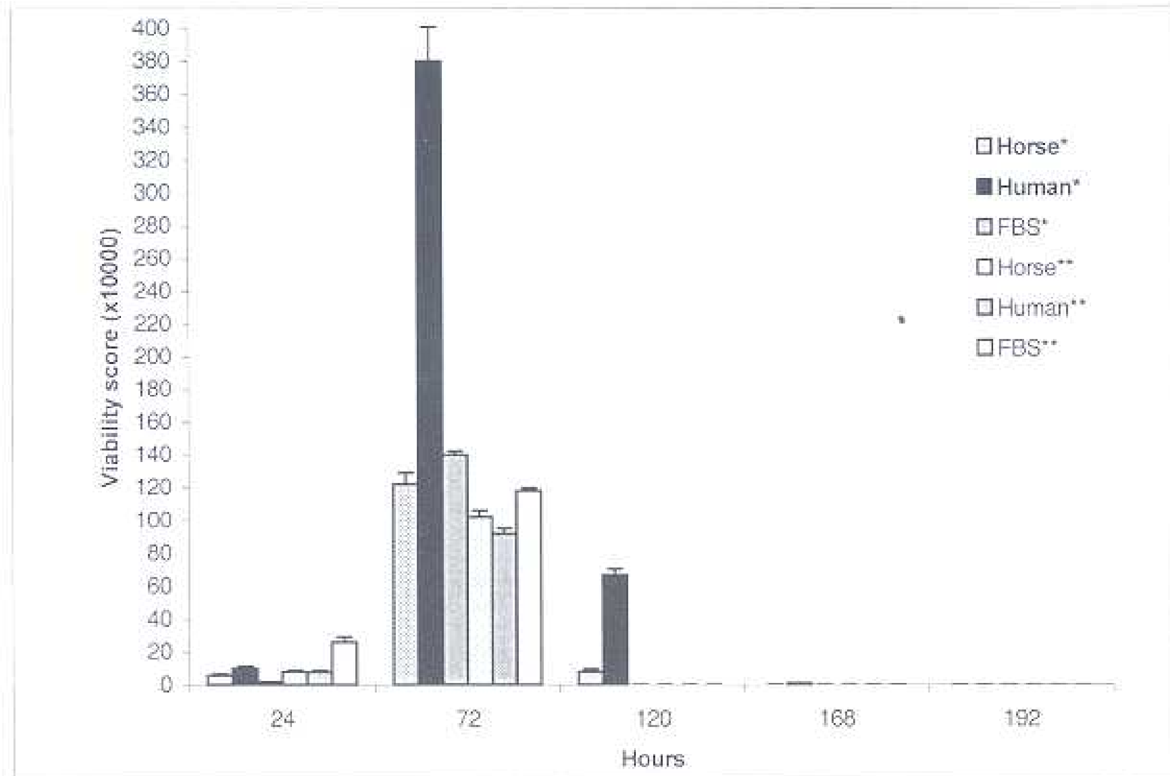

Figure 1. Comparison of the growth rates in different sera of two isolates of *T.vaginalis*
** Clinical isolate 1; ** Clinical isolate 2*

<u>Finding Related to Clinical Isolate 2:</u> There was no difference between the horse and the human sera in each counting period, when the effect of the clinical isolate 2 on the growth rate was examined. After 24 and 72 h, FBS was found to be more effective than both the horse and the human sera ($p< 0.05$, $p< 0.05$, respectively).

(In the comparison of the sera, Mann-Whitney U test was used as a statistical analysis Table II, Figure 1).

<u>Comparison of Clinical Isolate 1 with 2:</u> The clinical isolate 1 with human sera reproduced more than the isolate 2 ($p< 0.005$) after 72, 120 and 168 h, while the clinical isolate 1 with horse sera reproduced more than the isolate 2 ($p< 0.005$) after 120 h. At the end of 24 h, the clinical isolate 2 with FBS grew more than the isolate 1 ($p< 0.005$).

(In the comparison of the sera, Mann-Whitney U test was used as a statistical analysis) (Figure 1).

DISCUSSION

In media previously identified in studies carried out on the reproduction of a number of protozoa in culture environments under optimum conditions, certain changes aimed both to enhance the living conditions of the parasite and to reduce the cost of the media were made and sometimes the two media were combined often with considerably successful results (12, 18-20). It has been reported that mammalian sera or various sera fractions should be in a medium environment in studies aiming at reproduction of *T. vaginalis* in different axenic media (5,21,22).

Gelbert et al, (23) reported that modified Diamond prepared with horse serum was quite successful

in determining a very small number of parasites and that this particular parasite could stay alive in this medium for 168 h. In our study, we have determined that the growth properties of both *T. vaginalis* isolates in the modified Diamond medium with human, horse and fetal bovine sera were different. We have observed that the best reproduction was obtained with the horse sera in the clinical isolate 1 long as 168h, with the FBS in the clinical isolate2. The longest survival period was achieved in the 1st clinical isolate with the human sera. We have also established that reproduction after 24, 72, 120 and 168 h in the 1st clinical isolate with the human sera which was higher compared to the media with other sera.

In addition to studies stating that *T. vaginalis* reproduces quite well in TYI-S 33 medium containing 5-20 % bovine serum (22); there are also other studies claiming that the growth rate in the TYI-S 33 medium not containing bovine sera could be greater compared to the one with bovine sera (21). While bovine sera was used in some of the studies (22); FBS was used in some others (24). When we used FBS in the modified Diamond medium, we have established that this medium was more effective on reproduction in the 2 nd clinical isolate after 24 and 72 h.

The variability of host- parasite association totally affects the growth rates of each clinical isolate in culture (25,26). The fact that the best reproduction in our study was achieved by different sera in the 2 nd clinical isolate in which exactly the same medium was used is remarkable and worthy of attention.

In this study, we have determined that the human sera could be used at least as successfully as horse sera described in modified Diamond medium that had a positive effect on the live span of the protozoon. Considering the cost of obtaining horse serum commercially, it seems more appropriate to use the human sera in the Diamond medium since it can be produced easily under laboratory conditions.

In conclusion, we have come to believe that different clinical isolates may yield different results in a medium under standard conditions and that the difference between the sera added to the medium can affect the growth rate and life span of the parasite.

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