The effect of resorcinol on bovine spermatozoa parameters in vitro

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Short title: Resorcinol affects spermatozoa parameters in vitro

Summary

The goal of this study was to observe the effect of resorcinol on motility, viability and morphology of bovine spermatozoa. The semen was used from six randomly chosen breeding bulls. Ejaculate was diluted by different solutions of resorcinol in 1:40 ratio. Samples were divided into 7 groups with different concentrations of resorcinol (Control, RES1 – 4 mg/ml; RES2 – 2 mg/ml; RES3 – 1 mg/ml; RES4 – 0.5 mg/ml; RES5 – 0.25 mg/ml and RES6 – 0.125 mg/ml). Motility of spermatozoa was detected using CASA method at temperature of 37°C in time periods 0, 1, 2, 3, 4 hours from the start of the experiment. Significant motility differences between all groups except control and RES6 with difference of 5.58%, as well as between RES1 and RES2 groups with difference of 2.17% were found. Progressive motility had the same significant differences. Spermatozoa viability (MTT test) decreased compared to control in all experimental groups during the entire duration of experiment. Observing morphologically changed spermatozoa, no significant changes were observed and a higher percentage of spermatozoa with separated flagellum in all experimental resorcinol groups compared to control were detected. Also, increased number of spermatozoa with broken flagellum, acrosomal changes and other morphological forms in the group with the highest concentration of resorcinol (RES1) were found. Results of our study clearly show negative effects on motility parameters of spermatozoa which depend on concentration, cultivation temperature and time period.

Keywords

Resorcinol, Spermatozoa, Bovine, Motility, Viability, Morphological Changes

Introduction

Resorcinol is an organic substance from the group of benzenediols. It is 1,3-bensenediol with chemical formula $C_6H_4(OH)_2$. It is a colorless crystal substance and crystals of benzene. It is well dissolvable in alcohol and ethers and is not soluble in chloroform or H_2S. It oxidizes easily especially in alkaline environment (Cervinka et al., 1991). Resorcinol (CAS number 108-46-3) is solid at room temperature. It has use in rubber industry, manufacture of wood adhesives, flame retardants, UV stabilizers and dyes (Schmiedel and Decker, 2000; EC, 2002).

Resorcinol possibly acts as an endocrine disruptor with thyroid effect (WHO, 2006; CEHOS, 2012). It appears to inhibit thyroid peroxidase and affects iodine uptake (Tukes, 2017). When used dermally to cure skin ulcers it results into hypothyroidism (Lynch et al., 2002). Additionally, it causes skin sensitization via contact (WHO, 2006) though rarely in humans. It is shown by pharmacokinetic studies that resorcinol is quickly metabolized when absorbed orally, dermally or subcutaneously – with excretion urinally in the form of glucuronide and sulphate conjugates (WHO, 2006).

Human data are limited but more than eighty percent of orally supplemented resorcinol is excreted by urine within 24 hours in rats (Kim and Matthews, 1987). Resorcinol concentrations in biological fluids have been rarely reported. An analysis method for free resorcinol in human plasma and urine was developed (Yeung at al., 1981). Free and conjugated resorcinol in plasma and urine after 2% solution´s continuous application to the skin of three persons over the course of four weeks with the daily dose of 12 mg/kg bw was measured (Yeung et al., 1983). All plasma levels were below the detection level of 100 µg/l. The urinary concentration was 4800-33700 µg/l after 2 weeks of application and 1600-8400 µg/l after four weeks.

Resorcinol is a frequently used hair dye (Goebel et al., 2019). It is surprising that no biological monitoring studies have been carried out on exposure to humans. Most relevant human studies are likely to be ones of personal care products (Yazar et al., 2009, 2012; Hamann et al., 2014). The use of resorcinol in hair dyes was evaluated by the EU but because of insufficient data, risk assessment could not be performed (SCCP, 2008). Resorcinol is also a major metabolite of tannic acid (Nakamura et al., 2003; EFSA, 2014), that occurs in food and beverages. It has use as anti-browning food additive to fresh and frozen crustaceans (EFSA, 2010) and has also been found in cigarette smoke (WHO, 2006; Vaughan et al., 2008). Thus, it is likely that resorcinol would be found in the urine of the general population. The aim of this study was to analyze possible effects of resorcinol on bull spermatozoa as a cell model and to find the effects on cell structure (viability and morphology) and function (motility) *in vitro*.

Material and methods

Experimental design

In the study ejaculates of six randomly chosen breeding bulls from insemination station in Lužianky (Slovenské biologické služby, a.s.) were used. After the collection samples were transported to the laboratory using thermos to maintain the temperature. Thereafter the semen samples were diluted with different concentrations of resorcinol (Table 1). Experimental concentrations of resorcinol were set based on previous studies *in vivo* and *in vitro* (Skowroń and Zapór, 2004; Welsch et al., 2008; Rafajova, 2011).
Samples were cultured at the temperature of 37°C and the measurements were done in five different time periods (0, 1, 2, 3 and 4 hours).

Determination of spermatozoa motility

To evaluate the motility of spermatozoa the CASA system was used (Computer – Assisted Sperm Analysis), SpermVision program (Minitube, Tiefenbach, SRN) with microscope Olympus BX 51 (Olympus, Japan). Each sample was placed in Makler Conting Chamber with depth of 10 μm (Sefi-Medical Instruments, Haifa, Israel) and was afterwards placed in the microscope (Slanina et al., 2013; Krockova et al., 2016).
Using the bovine specific set following parameters in each sample were observed: MOT – motility of spermatozoa (%); PRO – progressive motility of spermatozoa (%); DAP – distance average path (μm); DCL – distance curved line (μm); DSL – distance straight line (μm); VAP – velocity average path (μm/s); VCL – velocity curved line (μm/s); VSL – velocity straight line (μm/s); STR – straightness; LIN – linearity; WOB – wobble; ALH – amplitude of lateral head displacement (μm) and BCF – beat cross frequency (Hz) as described previously (Tvrda et al., 2015; Adamkovicova et al., 2016; Halo et al., 2019). Each CASA measurement evaluated parameters of motility were calculated from at least seven different fields in the Makler Counting Chamber.

Determination of spermatozoa viability

Viability of bovine spermatozoa was evaluated by the metabolic activity (MTT) assay after 0, 1 and 4 hours of culture. This colorimetric assay measures the conversion of 3-(4.5-dimetylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, USA) to purple formazan particles by mitochondrial succinate dehydrogenase of intact mitochondria of living cells. Formazan was measured spectrophotometrically by a microplate ELISA reader

(Multiskan FC, ThermoFisher Scientific, Finland). The data are expressed in percentage of control. Results from the analysis were collected during four repeated experiments for each concentration (Slanina et al. 2016; Kňazická et al., 2012; Tvrdá et al., 2012).

Evaluation of spermatozoa morphology

For analysis of spermatozoa morphology, samples were fixed with Hancock's solution and stained with Giemsa (Zemanova et al, 2007; Massanyi et al., 2004; Roychoudhury et al., 2010a; Roychoudhury et al., 2010b). All slides were analyzed at the magnification 500x. For each sample at least 500 spermatozoa were evaluated and the percentage determined of the following: separated flagellum, flagellum torso, knob twisted flagellum, small head, large head, flagellum ball, retention of cytoplasmic drop, acrosomal changes, and other pathological spermatozoa (teratoid spermatozoa; a spiral twisted flagellum; deformation of the mitochondrial part and others).

To evaluate the results gained from CASA analysis in different time periods a statistical program SAS 9.2 with the use of Enterprise Guide 4.2 (SAS 9.2) was used. The significance of differences was evaluated on significance level of $p<0.05$ (Miškeje et al., 2013).

Results

In our study we observed the effect of resorcinol on motility of bull spermatozoa under *in vitro* conditions in five different time periods (0, 1, 2, 3, 4 hours).
Motility of spermatozoa at the temperature of 37°C was significantly ($p<0.05$) affected by addition of resorcinol. The percentage of motile spermatozoa (MOT) in the control group control were between 74.97% (Time 0) and 59.07% (after 4 hours of cultivation). At the highest concentration of resorcinol, the motility was close to 0 (Table 2). With decreasing concentration of resorcinol, the motility increased. Significant ($p<0.05$) changes in RES1 – RES4 samples compared to control group at Time 0, 1, 2 were detected. After 3 and 4 hours in all experimental samples significant decrease was found ($p<0.05$). Similar tendency was detected for progressive motility of spermatozoa (PRO). With highest values noticed is group with lowest concentration of resorcinol (RES6) in time zero (77.09%). In RES1 – RES4 samples we have seen significant contrast against control group ($p<0.05$) (Table 2).
During observation of distance parameters (DAP, DCL, DSL) we have seen increase in average paths (μm) with decreasing concentration of resorcinol in time zero but also with increasing time we observed a decrease. Highest concentration of resorcinol (RES1) caused death of spermatozoa. We recognized significant changes in RES1 – RES5 groups compared with control group ($p<0.05$) (Table 3).
There was an increase in velocity (μm/s) with decreasing concentration of resorcinol in velocity parameters (VAP, VCL, VSL), (Table 4). In RES1 time zero we observed VAP being 0 μm/s and in RES6 (sample with lowest concentration of resorcinol) it was 79.48 μm/s.
Straightness (STR), linearity (LIN) and wobble (WOB) are evaluated in table 6. In these we can also observe negative effect of higher concentrations in samples. There were significant changes in straightness between RES1 – RES3 groups in comparison with control group ($p<0.05$). Linearity and wobble were significantly changed in RES1 – RES3 against control group ($p<0.05$).
Amplitude of lateral head displacement (ALH) showed significant difference between control group and RES1 – RES3 ($p<0.05$). In this case it was not confirmed that decreasing resorcinol concentration increased levels of ALH (Table 5).

Lowering the concentration of resorcinol caused an increase of beat cross frequency (BCF) but also with increasing time there was a decrease in each sample. We observed significant changes ($p<0.05$) between control group (control) and groups RES1 – RES5. BCF significantly decreased in some samples ($p<0.05$) which can be explained by toxic effect of resorcinol on spermatozoa. In RES2 samples (2 mg/ml) in time zero we registered BCF of 14.35 Hz, whereas after 3 hours the frequency was down all the way to 1.69 Hz. Higher concentration of resorcinol has negative effect on beat cross frequency (Table 5). Spermatozoa viability (MTT test) decreased compared to control in all experimental groups during the entire duration of experiment with significant differences ($p<0.05$) (Table 6). Observing morphologically changed spermatozoa we have not noticed significant changes, but we have observed a higher relative percentage of spermatozoa with separated flagellum in all experimental resorcinol groups compared to control (Table 7). Furthermore, we have seen increased amounts of spermatozoa with broken flagellum, acrosomal changes and other morphological forms in the group with the highest concentration of resorcinol (RES1).

Discussion

The reproductive ability and the semen quality of animal species can be affected by many factors, as age, stress, hormonal status, nutrition, toxins etc. and solves as a fine barometer for the estimation of various effects (Jankovičová et al., 2015; Vitku et al. 2015; Heráček et al. 2018; Saha et al. 2019; Jambor et al., 2019).

CASA method is used to objectively evaluate the motility parameters of human and animal spermatozoa (Massányi et al. 2008). Development of CASA analysis (Dott and Foster, 1979) was based on movement of spermatozoa head and was made to study the spermatozoa in more detail. Evaluation of spermatozoa motility is an important parameter in common examination of spermatozoa but also in experimental studies (Palacín et al. 2013). CASA method is affected by many factors and techniques - i.e. optics, software setup, amount of analyzed concentrations, types of samples, dilutions (Contri et. al, 2010).

Motility is one of the most important parameters to evaluate the quality of spermatozoa. Active motility is inevitable for fertilization (Yániz et al., 2000). Based on our results we can state that with longer cultivation period increases and with rising concentration of resorcinol motility of spermatozoa decreases. Motility in control samples without addition of resorcinol was from 0.16% to 77.60% based on time interval. With concentration of 2 mg/ml (RES2) in time 0 we detected motility of 7.5±6.71% and with concentration of 1 mg/ml the motility was 31.14±20.66% whereas Rafajova (2011) found out that percentage of motility after addition of 2 mg/ml of resorcinol was 78.95±6.80% in time 0 and in group with addition of 1 mg/ml was 80.47±8.99%. Significant decrease of motility in first group began after one hour ($p<0.001$) as well as in other time periods. Spectating morphological changes of spermatozoa, Blom (1977) states that ejaculate gathered from young Hereford bulls had 80-95% of total amount of abnormal forms of spermatozoa where the head was separated from the tail, but high percent of free tails were capable of movement. Morphological changes were also observed by Massányi et al. (2000) based on season of the year. They found out that head changes represented the most of pathological forms of spermatozoa (21.89%) and the changes to tails represented 59.11% of all morphological changes. Most occurrent abnormality was decapitation which mirrored 18.99%.

Slamečka et al. (2001) aimed their study towards spermatozoa taken from epididymis of rabbis and stated that total amount of pathological spermatozoa is on the level of 11.50 – 24.71% and found out the most common anomaly was retention of cytoplasmatic droplet. Analysis of morphologically changed sperm in boars shown that in total, there was 8.28% of pathological spermatozoa out of which the most common anomaly was head missing tail

(Massányi et al., 2004). Terawaki et al. (1991) also studied morphological changes in spermatozoa. Ejaculate was gathered from Holstein bulls and average of abnormal spermatozoa was 9.1%. Most anomalies were found in head (1.4%) and in different parts of tail (7.2%).

Dinardo et al. (1985) studied the effect of resorcinol on pregnant rats and its fetus. They supplemented resorcinol in dosage of 125, 250 or 500 mg/kg of body weight. Resorcinol caused insignificant decrease of pregnant rat mass in groups that were fed by 500 mg/kg. It did not have any toxic effect on embryos or effect on fetus. Kavlock (1990) studied 15 groups of pregnant rats which were fed by resorcinol in doses of 333, 667 and 1000 mg/kg of body mass. Resorcinol had certain embryotoxic and teratogenic effects on fetus. A slight decrease of weight was measured after 72 hours of resorcinol supplementation.

Resorcinol in insemination dose is not harmful from health point of view but it has negative effect on spermatozoa. Effects on organism are being noticed only with high dosage or with long time exposure (Rafajova, 2011).

Our results show decrease in spermatozoa quality parameters which should be induced by the effects of resorcinol by various modes of action. In relation to mitochondrial activity Skowroń and Zapór (2004) proved that resorcinol caused inhibition of mitochondrial activity with a reduction of 60-80%. Resorcinol also caused inhibition of 3T3 cell growth in concentrations above 1 $\mu g/cm^3$ after 72 hours of exposure. Another study described the mechanism of cytotoxicity of a new active 5-alkyl resorcinol on HepG2 and Hep3B human hepatoma cell lines (Barbini et al., 2006). The IC50 values were detected 13.12 and 12.45 μg/mL, respectively. After the 24 h culture both cell lines showed induced apoptosis, DNA fragmentation and condensed and fragmented nuclei. Similarly, to our findings, authors report cytotoxic effect.

Conclusion

The goal of this study was to observe the effect of different concentrations of resorcinol of motility parameters of bull spermatozoa at the temperature of 37°C. Evaluation method was CASA. Using this technique, we came to the conclusions that with longer cultivation period the motility of spermatozoa increases. Also, with increased concentration of resorcinol the motility of spermatozoa decreases. Motility in control samples without the addition of resorcinol was on the scale of 0.6% to 77.60% depending on time period in which it was measured. Similar trends were detected for cell viability and morphology. From the results we can sum up that higher concentrations of resorcinol (phenolic substance) significantly decrease observed parameters, therefore showing negative effect.

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Conflict of interest

Authors have no conflict of interest to declare.

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Table 1 – Experimental groups

Group	Volume of ejaculate (µl)	Resorcinol (mg)	Physiological saline (µl)	Dilution
Control	20	0	800	1:40
RES6	20	0.152	800	1:40
RES5	20	0.25	800	1:40
RES4	20	0.5	800	1:40
RES3	20	1	800	1:40
RES2	20	2	800	1:40
RES1	20	4	800	1:40

Table 2 – Spermatozoa motility and progressive motility (%) in different groups and time periods

Group/Time	Time 0		Time 1		Time 2		Time 3		Time 4	
Motility (%)										
	x	SD	x	SD	x	SD	x	SD	x	SD
Control	**74.98**	**18.00**	**70.76**	**32.56**	**72.02**	**14.27**	**71.90**	**17.1**	**59.07**	**10.95**
RES6	77.60	13.50	77.09	14.33	63.16	16.16	60.11*	12.60	44.10*	17.53
RES5	71.86	15.63	67.88	18.79	57.38	20.84	46.19*	14.75	30.53*	17.90
RES4	65.55*	20.19	54.37*	27.17	45.25*	29.66	37.62*	17.99	23.21*	11.39
RES3	31.14*	20.66	29.49*	25.80	20.01*	23.28	15.52*	12.54	5.38*	4.83
RES2	7.50*	6.71	1.80*	2.21	1.60*	2.89	1.01*	1.69	0.98*	1.72
RES1	0.37*	1.6	0.62*	1.33	0.29*	1.14	0.58*	1.21	0.16*	0.80
Progressive motility (%)										
Control	**71.54**	**18.27**	**68.63**	**32.38**	**67.16**	**14.58**	**68.58**	**17.12**	**56.00**	**11.14**
RES6	73.90	14.21	74.84	14.74	60.07	15.63	56.79*	13.52	40.64*	17.70
RES5	67.96	16.94	64.93	18.63	54.48	21.39	44.00*	15.37	28.15*	16.83
RES4	60.95*	19.80	50.53*	26.65	43.09*	29.22	35.76*	17.97	20.62*	10.51
RES3	26.34*	19.22	26.84*	24.61	18.64*	22.81	13.46*	11.23	3.98*	4.59
RES2	5.16*	6.7	0.55*	1.12	0.88*	2.11	0.15*	0.56	0.08*	0.40
RES1	0*	0	0*	0	0*	0	0*	0	0*	0

* - $p < 0.05$. Control - control, RES1 – 4 mg/ml; RES2 – 2 mg/ml; RES3 – 1 mg/ml; RES4 – 0.5 mg/ml; RES5 – 0.25 mg/ml and RES6 – 0.125 mg/ml

Table 3 – Spermatozoa distance parameters (in μm) in different groups and time periods

Group/Time	Time 0		Time 1		Time 2		Time 3		Time 4	
	Distance average path (μm)									
	x	SD	x	SD	x	SD	x	SD	x	SD
Control	**37.66**	**7.80**	**34.68**	**7.7**	**30.69**	**2.94**	**27.3**	**3.62**	**24.24**	**4.26**
RES6	35.02	8.71	36.23	5.87	30.22	2.80	28.57	4.46	24.83	4.38
RES5	31.24*	7.6	33.70	7.00	26.59	4.13	24.58	5.31	21.85*	7.30
RES4	25.20*	6.13	28.69*	6.83	24.58*	4.25	22.08*	4.4	21.63*	3.74
RES3	17.64*	6.86	18.20*	9.93	18.91*	9.76	15.46*	8.28	13.29*	11.15
RES2	10.73*	8.95	3.32*	6.77	3.03*	6.22	1.18*	4.38	0.31*	1.40
RES1	0*	0	0*	0	0*	0	0*	0	0*	0
Distance curved line (μm)										
Control	**69.37**	**11.47**	**66.73**	**13.33**	**61.78**	**5.93**	**57.41**	**8.31**	**49.12**	**9.96**
RES6	62.94	14.46	69.18	10.97	60.30	5.94	59.62	11.89	51.15	11.5
RES5	59.42*	11.19	62.77	13.53	54.04*	10.89	51.49	13.70	44.06	15.64
RES4	49.69*	9.81	55.30*	13.28	47.50*	9.72	43.21*	10.85	41.48*	7.7
RES3	35.22*	13.66	35.44*	19.76	35.81*	19.99	29.00*	15.87	22.39*	18.31
RES2	21.86*	18.34	5.57*	11.70	4.74*	10.36	1.64*	5.97	0.40*	1.92
RES1	0*	0	0*	0	0*	0	0*	0	0*	0
Distance straight line (μm)										
Control	**32.00**	**7.79**	**28.89**	**6.66**	**24.80**	**3.3**	**20.21**	**3.64**	**18.75**	**3.92**
RES6	30.82	8.11	31.12	5.94	24.26	3.6	21.88	4.5	17.81	3.63
RES5	26.73*	7.1	29.6	7.23	20.00*	3.14	17.56*	4.26	15.39	5.43
RES4	20.67*	6.46	23.62*	6.91	17.24*	4.51	14.88*	3.84	13.95*	3.46
RES3	12.62*	5.71	14.05*	8.18	12.12*	6.43	9.72*	5.57	9.00*	9.4
RES2	7.28*	6.30	2.16*	4.40	2.03*	4.10	0.55*	2.3	0.30*	1.35
RES1	0*	0	0*	0	0*	0	0*	0	0*	0

* - $p < 0.05$. Control - control, RES1 – 4 mg/ml; RES2 – 2 mg/ml; RES3 – 1 mg/ml; RES4 – 0.5 mg/ml; RES5 – 0.25 mg/ml and RES6 – 0.125 mg/ml

Table 4 – Spermatozoa velocity parameters path (in µm/s) in different groups and time periods

Group/Time	Time 0		Time 1		Time 2		Time 3		Time 4	
	Velocity average path (µm/s)									
	x	SD	x	SD	x	SD	x	SD	x	SD
Control	**85.83**	**18.50**	**79.74**	**16.30**	**69.42**	**6.82**	**60.08**	**9.5**	**53.51**	**9.68**
RES6	79.48	21.2	82.46	13.71	67.61	6.63	63.58	10.66	54.44	9.65
RES5	69.65*	15.84	75.71	16.88	58.95*	8.78	53.87	11.94	47.75	16.2
RES4	55.25*	13.62	64.07*	15.16	54.65*	10.11	47.95*	8.60	46.79*	7.90
RES3	38.00*	14.85	40.00*	21.83	41.12*	21.57	33.05*	17.83	29.25*	24.20
RES2	23.91*	20.4	7.19*	14.31	7.91*	15.83	3.43*	13.16	0.73*	3.31
RES1	0*	0	0*	0	0*	0	0*	0	0*	0
Velocity curvilinear line (µm/s)										
Control	**157.39**	**26.91**	**152.99**	**30.78**	**139.33**	**12.17**	**126.98**	**19.31**	**108.18**	**22.22**
RES6	142.12	34.01	157.26	25.54	134.43	13.57	132.25	27.58	111.90	23.61
RES5	131.99	24.80	140.57	32.12	119.50	22.82	112.37	30.22	96.03	34.10
RES4	108.68*	21.80	123.15*	29.16	105.27*	22.46	93.60*	23.31	89.75*	15.56
RES3	75.71*	29.37	77.50*	43.15	77.56*	43.37	61.90*	34.21	49.17*	39.96
RES2	48.68*	41.39	11.90*	24.46	11.87*	24.82	4.74*	17.71	0.93*	4.37
RES1	0*	0	0*	0	0*	0	0*	0	0*	0
Velocity straight line (µm/s)										
Control	**73.13**	**18.39**	**66.47**	**15.39**	**56.03**	**6.96**	**44.98**	**9.00**	**41.36**	**8.82**
RES6	70.12	19.61	70.82	13.72	54.26	7.11	48.67	9.60	39.06	8.6
RES5	59.62*	15.56	65.34	17.19	44.39*	6.91	38.50	9.57	33.67*	12.7
RES4	45.33*	14.21	52.74*	15.37	38.41*	10.40	32.31*	8.29	30.32*	7.56
RES3	27.20*	12.41	30.86*	17.91	26.37*	13.94	20.83*	12.7	19.83*	19.46
RES2	16.20*	14.4	4.74*	9.48	5.45*	10.91	1.52*	5.54	0.71*	3.19
RES1	0*	0	0*	0	0*	0	0*	0	0*	0

* - $p < 0.05$. Control - control, RES1 – 4 mg/ml; RES2 – 2 mg/ml; RES3 – 1 mg/ml; RES4 – 0.5 mg/ml; RES5 – 0.25 mg/ml and RES6 – 0.125 mg/ml

Table 5 – Additional spermatozoa parameters in different groups and time periods

Group/Time	Time 0		Time 1		Time 2		Time 3		Time 4	
	x	SD	x	SD	x	SD	x	SD	x	SD
Straightness										
Control	**0.84**	**0.04**	**0.82**	**0.04**	**0.80**	**0.04**	**0.74**	**0.05**	**0.76**	**0.04**
RES6	0.87	0.03	0.85	0.04	0.80	0.04	0.76	0.04	0.71	0.06
RES5	0.84	0.04	0.85	0.05	0.75	0.05	0.71	0.06	0.65	0.20
RES4	0.80	0.08	0.81	0.07	0.69	0.09	0.66	0.09	0.64	0.11
RES3	0.63*	0.24	0.61*	0.31	0.55*	0.24	0.50*	0.27	0.44*	0.33
RES2	0.43*	0.39	0.14*	0.28	0.15*	0.29	0.03*	0.13	0.05*	0.21
RES1	0*	0	0*	0	0*	0	0*	0	0*	0
Linearity										
Control	**0.46**	**0.06**	**0.43**	**0.03**	**0.40**	**0.03**	**0.35**	**0.04**	**0.38**	**0.03**
RES6	0.49	0.06	0.44	0.04	0.40	0.03	0.37	0.04	0.35	0.04
RES5	0.44	0.05	0.46	0.04	0.37	0.05	0.34	0.04	0.32	0.10
RES4	0.40	0.06	0.42	0.05	0.36	0.06	0.34	0.04	0.33	0.06
RES3	0.31	0.11	0.32	0.16	0.30	0.14	0.26	0.14	0.28	0.25
RES2	0.21	0.17	0.09	0.19	0.11	0.24	0.02	0.09	0.04	0.18
RES1	0	0	0	0	0	0	0	0	0	0
Wobble										
Control	**0.54**	**0.05**	**0.52**	**0.26**	**0.49**	**0.03**	**0.47**	**0.03**	**0.49**	**0.03**
RES6	0.55	0.05	0.52	0.03	0.50	0.02	0.48	0.03	0.49	0.04
RES5	0.52	0.04	0.53	0.03	0.49	0.04	0.48	0.04	0.46	0.14
RES4	0.50	0.04	0.52	0.03	0.52	0.05	0.52	0.07	0.52	0.06
RES3	0.45*	0.15	0.42*	0.22	0.47*	0.21	0.43*	0.22	0.41*	0.32
RES2	0.32*	0.25	0.14*	0.27	0.15*	0.31	0.05*	0.19	0.04*	0.19
RES1	0*	0	0*	0	0*	0	0*	0	0*	0

* - $p < 0.05$. Control - control, RES1 – 4 mg/ml; RES2 – 2 mg/ml; RES3 – 1 mg/ml; RES4 – 0.5 mg/ml; RES5 – 0.25 mg/ml and RES6 – 0.125 mg/ml

Table 6 – The effect of resorcinol on the viability (MTT test; %) of bovine spermatozoa after 0, 1 and 4 hours of cultivation

Group	x	SD	min	max	CV
Time 0					
Control	89.96	4.4	80.55	95.34	4.49
RES6	87.80	5.81	75.86	95.23	6.61
RES5	66.88*	11.64	48.83	91.17	17.41
RES4	13.86*	11.31	2.4	32.14	81.60
RES3	0.85*	1.32	0	4.16	156.12
RES2	3.78*	3.84	0	10.00	101.67
RES1	0	0	0	0	0
Time 1					
Control	82.61	14.1	57.40	98.66	16.96
RES6	67.56*	11.42	45.00	83.78	16.90
RES5	22.02*	7.9	11.53	37.20	32.21
RES4	1.10*	1.53	0	3.33	139.59
RES3	1.15*	1.40	0	3.33	121.48
RES2	1.99*	2.39	0	7.69	119.95
RES1	0	0	0	0	0
Time 4					
Control	85.25	7.29	71.42	95.23	8.55
RES6	64.54*	13.72	45.83	81.25	21.26
RES5	13.64*	10.28	2.70	30.00	75.36
RES4	0.82*	1.82	0	6.25	223.05
RES3	0.82*	2.10	0	7.69	254.17
RES2	0.57*	1.53	0	5.26	269.10
RES1	0.79*	2.97	0	11.11	374.17

* - $p < 0.05$. Control - control, RES1 – 4 mg/ml; RES2 – 2 mg/ml; RES3 – 1 mg/ml; RES4 – 0.5 mg/ml; RES5 – 0.25 mg/ml and RES6 – 0.125 mg/ml

Table 7 – Relative occurrence of spermatozoa morphological changes (%) in samples with different concentrations of resorcinol

Parameter / Group	**Control**	RES6	RES5	RES4	RES3	RES2	RES1
Separated flagellum	19.23	13.33	11.40	23.81	15.48	17.57	13.33
Flagellum torso	16.35	**20.00**	**28.7**	**31.75**	**27.38**	**20.27**	**20.00**
Knob twisted flagellum	4.81	10.67	0	7.94	0	6.76	0
Small head	7.69	6.67	8.77	0	11.90	10.81	**13.33**
Large head	7.69	6.67	0	0	0	6.76	**13.33**
Flagellum ball	9.62	9.33	15.79	12.70	15.48	13.51	6.67
Retention of cytoplasmic drop	9.60	6.67	7.2	15.87	5.95	6.76	**13.33**
Broken flagellum	12.50	20.00	7.2	7.94	11.90	6.76	6.67
Acrosomal changes	12.50	0	21.93	0	5.95	10.81	6.67
Other morphological changes	100	100	100	100	100	100	100
Total count	0	6.67	0	0	5.95	0	6.67

* - p< 0.05. Control - control, RES1 – 4 mg/ml; RES2 – 2 mg/ml; RES3 – 1 mg/ml; RES4 – 0.5 mg/ml; RES5 – 0.25 mg/ml and RES6 – 0.125 mg/ml

Figure 1 – Motility of spermatozoa during evaluation hours (in %)

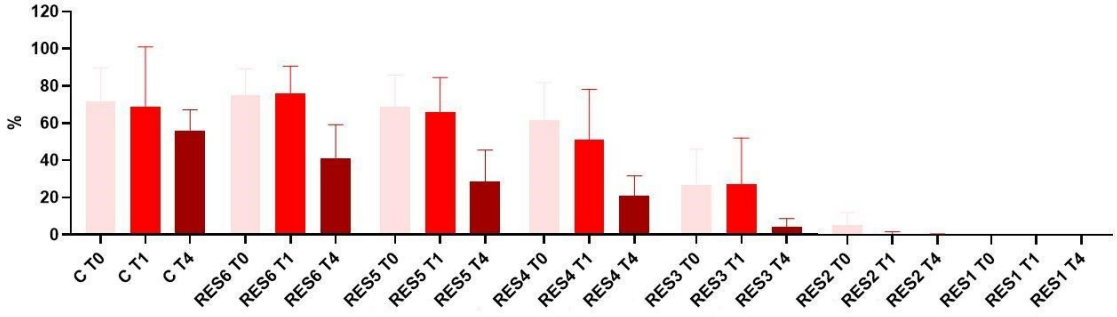

Figure 2 – Viability of spermatozoa during evaluation hours (in %)