

## THE ROLE OF PH VALUES IN PORCINE REPRODUCTIVE TRACTS OF MALE AND FEMALE INDIVIDUALS\*\*

T. Smiljaković<sup>\*1</sup>, S. Josipović<sup>1</sup>, O. Kosovac<sup>1</sup>, N. Delić<sup>1</sup>, S. Aleksić<sup>1</sup>, M. M. Petrović<sup>2</sup>

<sup>1</sup>Institute for Animal Husbandry, Autoput 16, 11080 Belgrade-Zemun, Serbia

<sup>2</sup>Ministry of Agriculture, Forestry and Water Management, Belgrade, Serbia

\*Corresponding author: [smiljakovic@gmail.com](mailto:smiljakovic@gmail.com)

\*\*Original scientific paper

**Abstract:** For a long time, in practice, and science, has been known that pH values of sperm and vagina are important for successful fertilization. In this investigation this fact was confirmed, and the goal was to investigate the role of pH values through whole reproductive tract of male and female individuals: testis, rete testis, epididymis, ductus deferens, Cowper's gland, vesicula seminalis, prostata, corpus cavernosus, corpus spongiosus, epithel tissue of penis tube, sperm, vagina, uterus, horn of uterus, oviduct, fimbriae ovarica, ovarium, follicular fluid. Measurement was performed in reproductive active males as well as before and after ovulation in females. Porcine reproductive tracts (per 15 female and male individuals) were collected from institute's slaughterhouse, immediately post mortem dissected, homogenised and pH values were measured (according to method *Rede&Rahelić* (1969)). Ovarium and follicular liquid have the highest pH values (7,4) in females, but a small peak in preovulatory oviduct is also present and corresponded to pH of sperm of reproductive fully active male individuals (pH=app.7,2). After fertilization pH in surrounding of zygote (through depolarisation of its membrane) in oviduct, and zygote which then has external decreased pH value moves to less pH values regions by the same principle, that means to uterus, (pH between 7,2 (horn) and 7,07(cervix)) in postovulatory female reproductive tract, where nidation of blastocyst occurs. This investigation could help to elucidate knowledge about reproductive physiology in vivo, giving importance to role of pH values along reproductive tract of male and female individuals.

**Key words:** reproductive tract, male, female, pH value, oviduct, sperm

## Introduction

For a long time it is known that pH values of sperm and vagina are important for successful fertilization. In the last decades, by introduction of methods for artificial insemination and in vitro fertilization in optimization of conditions for these methods, pH values of media for sustaining spermatozoa and oocytes in vitro, and capacitation of sperm had important role. These techniques had to enable conditions *in vitro* as much as possible like conditions during *in vivo* successful fertilization. Therefore in this investigation pH values of whole male and female reproductive tracts were measured directly post mortem, with goal to improve *in vivo* as well as *in vitro* fertilization in mammals. Our model system is porcine reproductive tract, but investigations would be done in other domestic animals as well, to specify conditions for each species of interest. In sight of new investigations in the field of reproduction there were many experiments which confirmed the ability of sperm features to influence hormonal and immunological status of female reproductive tract. For cytokine production in endometrium and progesterone synthesis in ovaries is confirmed that they depend on presence of sperm plasma, *S'O Leary et al.* (2004, 2006). In this paper will be described the role of the pH values along reproductive tracts in pig, and some recommendations will be done according to knowledge in the field of reproduction nowadays.

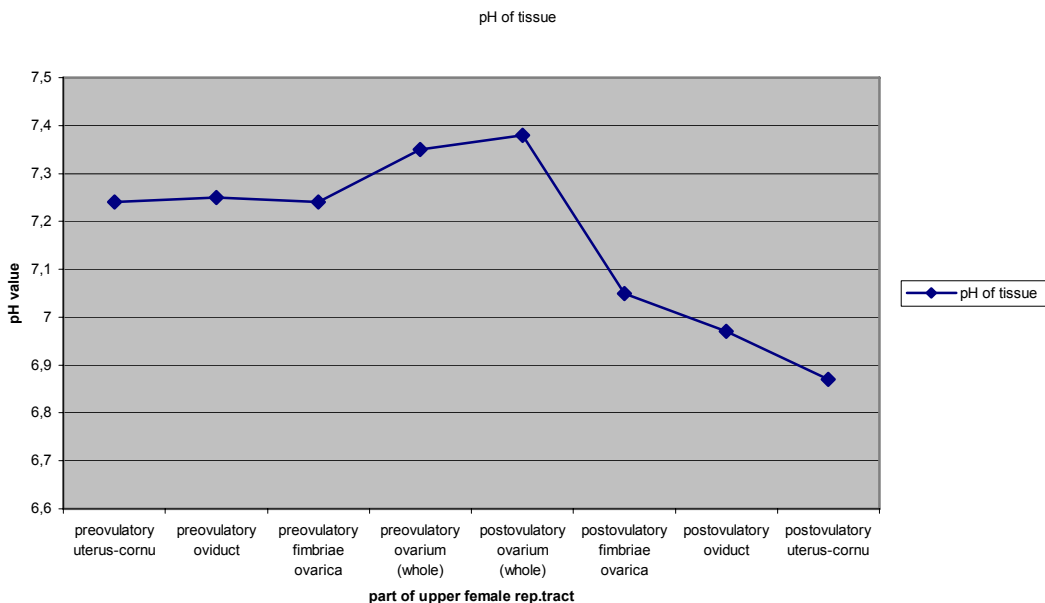
## Material and methods

Porcine (breed Landrace) 15 male and 15 female reproductive tracts were collected from institute's slaughterhouse, dissected, homogenised and according to method (*Rede and Rahelić, 1969*) pH values were measured. 1g of solid homogenized tissue or 1ml of liquid tissue were added to 10ml of distilled water. After 1h pH values were measured on pH-meter (Consort, C380, Belgium). Statistics was done by Microsoft Excel software.

## Results and discussion

On Figure 1 is visible that in upper part of preovulatory female porcine reproductive tract, the pH values are increased from horn of uterus until ovary, with a small but significant peak in oviduct, where fertilization occurs. On the same figure is depicted that late postovulatory (yellow bodies are not present yet), the pH values from horn of uterus till ovary gradually increases, without the peak in oviduct. The pH value of small but significant peak in preovulatory oviduct, bounded by two lower values, could be reason for waiting oocytes for

spermatozoids and vice versa ,on this spot, till they found each other and fertilization occurs. It is also significant that pH of adult sperm corresponds to upper part of female reproductive tract, so we could suppose that, "similis similiae gaudet", oocytes and spermatozoids by iso-pH value find each others. But, this should be confirmed in following studies. Capacitation of sperm, for decades, has been used by scientists in *in vitro* techniques, by changing pH of frozen sperm to pH=7,2 just before successful fertilization, could be confirmation for this hypothesis.



**Graph 1. pH value has a small peak in preovulatory oviduct. Postovulatory pH values are finally decreased, from ovarium until uterus**

**Grafikon 1. pH vrednost ima povećanje u preovulatornom jajovodu. Postovulatorno, pH vrednosti postepeno opadaju, od jajnika do materice**

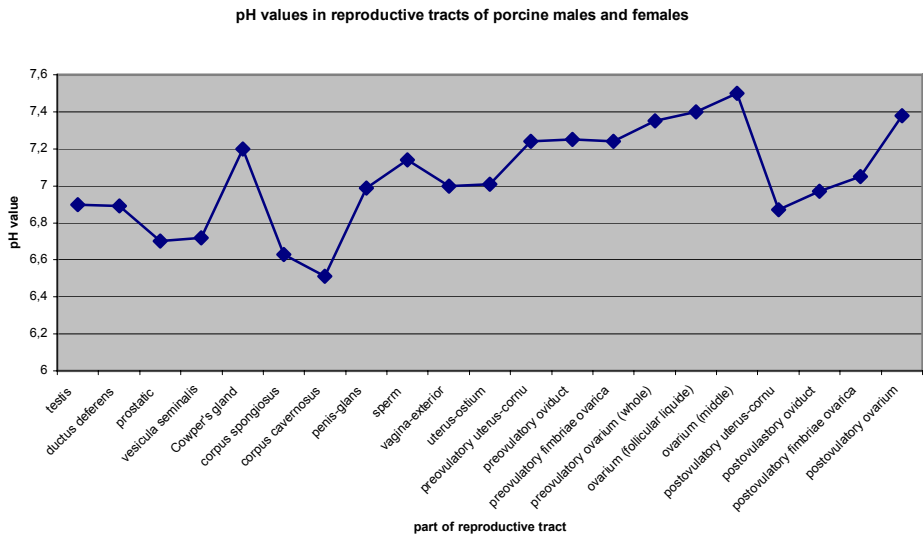
organ or tissue /organ ili tkivo	pH (average values)/ pH (prosečne vrednosti)	Variance/varijansa
testis	6,9	0,000225
ductus deferens	6,89	0,01075556
prostatic	6,7	1E-04
vesicula seminalis	6,72	2,5E-05
Cowper's gland	7,2	0,0049
corpus spongiosus	6,63	0,02382222
corpus cavernosus	6,51	0,024025
penis-glans	6,99	0,007225
sperm	7,14	0,02646667
vagina-exterior	7	0,010075
uterus-ostium	7,01	0,00915556
preovulatory uterus-cornu	7,24	0,01848889
preovulatory oviduct	7,25	0,02686667
preovulatory fimbriae ovarica	7,24	0,01108889
preovulatory ovarium (whole)	7,35	0,031784
ovarium (follicular liquide)	7,4	0,0256
ovarium (middle)	7,5	0,0017
postovulatory uterus-cornu	6,87	0,02555
postovulatory oviduct	6,97	0,02856875
postovulatory fimbriae ovarica	7,05	0,00271875
postovulatory ovarium	7,38	0,010325

**Tab.1. pH values throughout male and female (upper part shown preovulatory and postovulatory) reproductive tracts**

**Tabela 1. pH vrednosti duž muškog i ženskog (gornji delovi prikazani preovulatorno i postovulatorno) polnog trakta**

In Table 1 are presented averages of measured pH values through whole male and female reproductive tract, with beginning value of testis and ending value of parts of ovary. Sperm and follicular liquid of ovary are also investigated, and presented in table1. pH value of adult ejaculated sperm correspond to pH value of uterus cervix, wherefrom sperm moves by self energy, energy of muscles in corpus cavernosum and corpus spongiosum of penis, and as we suppose to more basic pH values of upper part of female reproductive tract which correspond to pH value of Cowper's gland which was proved to have a role in increasing of pH of sperm especially taking account that urinary and sperm tube in penis is the same, and urine in bladder has a

significant lower pH value (according to our results: 5,9). Results are presented in Figure 2, for better visualization.



**Graph 2. pH values throughout male and female (preovulatory and postovulatory) reproductive tract. It is obvious that the highest pH value in masculine reproductive tract is in Cowper's gland (see text for details), enabling sperm to "travel and find" oviduct. Grafikon 2. pH vrednosti duž muškog i ženskog polnog trakta. Očigledno je da u muškom polnom traktu Kauperova žlezda omogućava da sperma putuje i nađe jajovod.**

The pH values of upper (uterus-cornu (horn), oviduct and Falopian tube) preovulatory in females reproductive tract are higher than sperm pH (Tab.1). On the contrary, upper parts of reproductive tract of females are at declined values of pH (Fig. 1) late postovulatory that we proved in this experiment (Fig 1) and could indicate a non-attractive area for sperm moving in postovulatory female reproductive tract, especially taking into account contemporary ciliary waves to downstream of oviduct epithelium – reotaxism. Mobility is supported to upper female reproductive parts also by antiperistaltic contraction of uterus and oviduct. In vivo, investigations with intrauterine-oviduct sound, *Wähner & Geyer(2007)*, with small pH meter could give precise information, and could be useful in medicine and veterinary medicine for eventual diagnostic of reasons for infertility, which could be medicated by application of pH adjusting medicaments in uterus-cervical area before copulation or adjusting pH of sperm and uterus to oviduct in artificial

insemination. The pH values of sperm from pubertal gilts in our experiment were 7,49, and from adults 7,14. Mobility of spermatozooids depends on pH value of surrounding area: it is lower in acidic, higher in alkali and the best in low alkali pHs (like in our measured female reproductive tracts), and as *in vitro* experiments confirmed, *Pantić* (1990). According to results, capacitation of sperm, we suppose, activates ATP dependant H<sup>+</sup> pumps, *Lodish et al.* (2001) in acrosome membrane, decreases pH of acrosome liquide enabling moving of spermatozooids trough more and more basic gradient in female reproductive tract, till oviduct where pH value of sperm (spermatozoa surrounding) corresponds to pH value of oviduct around ovulation time (small peak 'waiting' surrounding of oocyte). These results are confirmed by investigations of sperm distribution, by dissection and haemocytometry of spermatozooids, in upper parts in the reproductive tract of sows after interuterine insemination and conventional artificial insemination in which was shown that oviduct is reservoir of sperm around ovulatory time, *Sumransap et al.* (2007). Also, after natural insemination of gilts, the number of spermatozoa recovered in the uterus diminished significantly during the first 12 hours after mating, but in the uterotubal junction and oviduct doesn't vary significantly after 2, 6 and 12 hours after mating, *Viring S & Einarsson S* (1981). After fertilization pH in surrounding of zygote (through depolarisation of its membrane (*Müller & Hassel* (2003)) in oviduct is decreased, and zygote which then has external pH, by the same principle, moves to less pH values regions, that means to uterus, (pH between 7,2 (horn) 7,07(cervix)) in postovulatory female reproductive tract, where nidation of blastocyst occurs. In sight of *in vitro* techniques, we recommend a passage of spermatozooids through pH values in media which correspond found pHs from seminal vesicle downstream along tube of masculine reproductive tract.

## Conclusion

Our preliminary data could explain how pH values of tissues through porcine male and female reproductive tracts support moving of spermatozooids through male reproductive tract, to capacitation of spermatozooids *in vivo*, enable coitus, and moving of spermatozooids through female tract to oviduct where *in vivo* fertilization occurs, changing pH of surrounding area by depolarisation, *Müller and Hassel*, (2003). Also, pH value is important for sustaining of oocyte in oviduct, and after fertilization downstream moving of zygote, morula and blastocyst to the uterus, where nidation occurs *in vivo*. This investigation elucidate the reproductive physiology *in vivo*, and could be important to applications in reproduction in agriculture and veterinary medicine, as well as model system for reproduction of other domestic mammalian animals

and breeds, reproduction of the endangered mammalian species using artificial insemination or *in vitro* techniques and as model system for human andrology and gynaecology.

## Acknowledgements

We wish to thank Mrs Miroslava Maksimović, Mrs Snežana Trenkovski, Mrs Ljiljana Stojanović and Mrs Sandra Drakić for expert technical assistance and Mr Saša Pejčić for information about reproduction status of examined animals on the farm, as well as technicians from institut's farm and experimental slaughterhouse for help during the work. This work was financially supported by Serbian Ministry of Agriculture, Forestry and Water Management. T.Smiljaković was a fellow of the German Academic Exchange Service (DAAD) during doctoral studies with supervision of Dr.Wolfgang Tomek, at Department for Reproduction,in FBN-Dummerstorf, and at Biological faculty, Rostock University, and these supports are gratefully acknowledged.

## Uloga pH vrednosti u muškom i ženskom reproduktivnom traktu svinja

*T. Smiljaković, S. Josipović, O. Kosovac, N. Delić, S. Aleksić, M. M. Petrović*

### Rezime

U praksi i nauci je odavno poznato da je pH vrednost sperme i vagine od značaja za uspešnu oplodnju. U ovom istraživanju je to potvrđeno, a cilj istraživanja je bio da se ispita uloga pH vrednosti duž celog polnog trakta mužjaka i ženki svinja: od testisa, epididimisa ductus deferens, Kauperove žlezde, semene kesice, prostate, corpus cavernosus i corpus spongiosus penisa, epitelnog tkiva cevi penisa, sperme vagine, materice, rogova materice, jajovoda, fimbrie ovarica, jajnika, do folikularne tečnosti jajnika. Merenje je izvršeno kod polno aktivnih mužjaka i ženki (pre- i postovulatorno). Polni trakt svinja (po 15 ženskih i muških jedinki) je uzet iz eksperimentalne klanice instituta, odmah post mortem izvršena je disekcija, homogenizacija čvrstih tkiva i merene su pH vrednosti (prema metodi Rede i Rahelić (1969)). Jajnik i folikularna tečnost jajnika imale su najvišu pH vrednost od 7,4, dok je mali ali značajan porast pH

vrednosti u preovulatornom jajniku odgovarao pH vrednosti sperme polno aktivnih mužjaka (pH oko 7,2). Nakon oplodnje u okolini zigota (putem depolarizacije membrane) u jajovodu se snižava pH vrednost, što ostaje za dalja istraživanja, i zigot koji tada ima spoljašnju smanjenu pH, kreće se, potpomognut pokretima trepljastog epitela, ka regionu niže pH vrednosti, odnosno materici (u rasponu pH od 7,2 u rogu materice do 7,07 u cerviksu) u postovulatornom polnom traktu ženki i u materici se odvija nidacija odnosno ugnježdavanje blastule ukoliko je oplodnja uspesna. Ova istraživanja bi mogla da rasvetle znanja o polnoj fiziologiji *in vivo*, dajući značaj pH vrednostima duž polnog trakta muških i ženskih jedinki.

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