

ORIGINAL ARTICLE

Medicine Science 2020;9:619-24

Association between some hematological parameters and in vitro fertilization outcomes **Ayşe Nur Yildirim**,  **Osman Nuri Keles***Atatürk University, Faculty of Medicine, Department of Histology and Embryology, Erzurum, Turkey*

Received 25 April 2020; Accepted 19 May 2020

Available online 08.08.2020 with doi: 10.5455/medscience.2020.04.055

Abstract

The aim of this study was to determine the effects of some hematological parameters on fertilization success, embryo quality and pregnancy rates in the in vitro fertilization (IVF) treatment. 50 infertile couples aged 22-46 years who were admitted to Atatürk University Research Hospital for IVF treatment were included in the study. The number of fertilized oocytes, embryo quality, clinic pregnancy rate were determined and compared with hematological parameters. In the male patients, the platelet distribution width (PDW) value was negatively correlated with the fertilization rate ($r_p = -0.341^*$; $p = 0.015$). A negative relationship between the transplantable embryo rate and the mean corpuscular volume (MCV) value was observed ($r_p = -0.320^*$; $p = 0.024$) in the male. While the hemoglobin (HGB) value was negatively correlated with the fertilization rate ($r_p = -0.332^*$; $p = 0.019$), the red cell distribution width (RDW) value was positively correlated with the fertilization rate ($r_p = 0.359^*$; $p = 0.010$) in female patients. In addition, significant correlations were found between PDW value both good quality embryo rate (regression coefficient: -19.808 , $p = 0.007$) (corrected as -19.808) and transplantable embryo rate (regression coefficient: -16.855 , $p = 0.033$) (corrected as -16.855) in female patients. In conclusion, changes in PDW values of female patients significantly affect embryo quality, may result pregnancy failure and abnormal embryo and fetus development during pregnancy.

Keywords: Fertilization in vitro, embryo, pregnancy, blood cell count**Introduction**

Infertility is a common human health problem [1] and is defined as the fact that pregnancy cannot occur in couples of reproductive age even though they do not use any contraceptive method and have at least one year of regular sexual intercourse [2]. Recently, there have been very important developments in the diagnosis and treatment of infertility, and many couples who have been considered impossible to possess children have been able to have children due to new methods. However, making the correct diagnosis, conducting a detailed examination and applying the right treatment methods are the most important elements in the success rate of increasing pregnancy rates [3]. Despite all these developments, infertility affects about 10% to 15% of couples in childbearing age in industrialized countries [4]. The majority of these couples apply ART (assisted reproductive technology) or IVF treatment [5]. In couples undergoing IVF treatment, the problem of infertility may be due to male, female or unexplained

reasons [6]. Among the most common causes of female infertility are problems with ovulation, uterine injuries, problems with the fallopian tubes and cervix, immunological and endocrine factors, and genetic causes [7]. Male infertility often results from abnormal sperm function, a decrease in semen quality and quantity and genital tract obstruction that prevents the excretion of sperm [8].

IVF is an important method used in the treatment of infertility when other assisted reproductive methods are unsuccessful, it involves procedures such as egg retrieval from the ovaries, a single sperm injection into each egg with ICSI (intracytoplasmic sperm injection) and the development of blastocyte from fertilized egg in a culture medium and the blastocyte transferred to the woman's uterus [9]. Success in this procedure is to achieve a high pregnancy rate or healthy fetus due to correct ICSI application and quality embryo selection [10]. Also, many factors such as age, weight, hormonal status, oocyte quality, ovarian reserve quality, pregnancy-appropriate endometrium structure and health status of the candidate, affect the IVF success [11].

The complete blood count (CBC) is a blood test used to evaluate the numerical and structural changes in the red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs) [12] and

*Corresponding Author: Osman Nuri Keles, Atatürk University, Faculty of Medicine, Department of Histology and Embryology, Erzurum, Turkey, E-mail: onkeles29@yahoo.com

detect a wide range of disorders, including anemia, infection and leukemia, clotting problems, immune system disorders [13]. Some hematological parameters associated with systemic inflammation has been shown to may cause adverse effects on the gonad quality leading to consequent both male [14] and female infertility [15]. However, there have been no reports showing a relationship between all hematological parameters and IVF outcomes in infertile couples without any disease. Therefore, we investigated whether there is a relationship between the levels of hematological parameters in patients within reference ranges for blood tests, and fertilization rate, emryo quality which are IVF outputs and pregnancy rate.

Materials and Methods

In our study, fifty couples aged 22-46 years who applied to IVF treatment at Atatürk University Research Hospital IVF Center were examined. The necessary permission was obtained from the Ethics Committee of Atatürk University Faculty of Medicine (24.07.2014/8).

Oocyte collection was performed by the gynecologist physician under anesthesia. With the help of the Oocyte Pick-up (OPU) needle (Gynetics; Belgium), the follicles were aspirated and the aspirated follicle fluids were transferred to 14 ml tubes (Falcon, BD France). The follicle fluid taken by aspiration was poured into the petri dish (Falco; USA) and oocytes were selected and taken under a stereo microscope (SZ 61 Olympus, Japan). Oocytes were selected as indicated by Brackett and Zuelke [16]. Hyaluronidase enzyme (Hyase-10X, Vitrolife, Colorado, USA) was added to the selected oocytes and separation process was performed from the cumulus cells around the oocytes with the help of a 135-175 mm pipette. The oocytes separated from the cumulus cells were transferred into a special culture medium, placed in the incubator and kept until ICSI time. Metaphase II stage oocytes were subjected to ICSI treatment.

Male patients were asked for a semen sample after 3-5 days of sexual abstinence. After taking the sample, the semen sample was expected to be liquefied for 30 minutes. After the samples were examined in terms of semen volume, viscosity and pH, morphological evaluations were performed. The semen sample that was mixed by pipetting was placed on the Makler Chamber and examined at 20X magnification under the light microscope and sperm number and morphology was evaluated according to World Health Organization (WHO)'s criteria. Sperm morphology was evaluated according to Kruger's criteria [17]. Sperm motility was evaluated between 1 (no movement) and 4 (fast forward motility) in accordance with WHO criteria. The sperm sample was prepared for ICSI treatment and kept at 37°C for 30 minutes in the incubator.

In the ICSI, the sperm were taken into the a glass micropipette by the tail after the tail of the spermatozoon was broken and inserted into the vitellus of the mature oocytes (MII) and the spermatozoon was then released into the oocyte with ICSI micromanipulator. Approximately 17-18 hours after ICSI, it was checked whether the presence of two pronuclei or fertilization (Figure 1A and 1B). Also, 24 hours after fertilization, zygotes that began to divide were defined as cleavager, and those that did not begin to divide as non-cleavager. Later, embryos were examined by light microscopy on the second and third days and evaluated according to their division and fragment status (Figure 1C and 1D). The structure of blastomeres, symmetry, cell number, cleavage status and fragmentation rate in the cytoplasm

were the parameters that are taken into account when scoring. The reason of the these assessments was to choose the best embryo and increase pregnancy success. When making the evaluation, it was carried out considering the scoring system of Baczkowski [18]. After the assessment was completed approximately 48-72 hours after the ICSI procedure, embryo transfer was performed by the gynecologist under the guidance of ultrasonography. 10-12 days after embryo transfer, whether the embryo implanted or not was determined by measuring the β -hCG value in the blood.

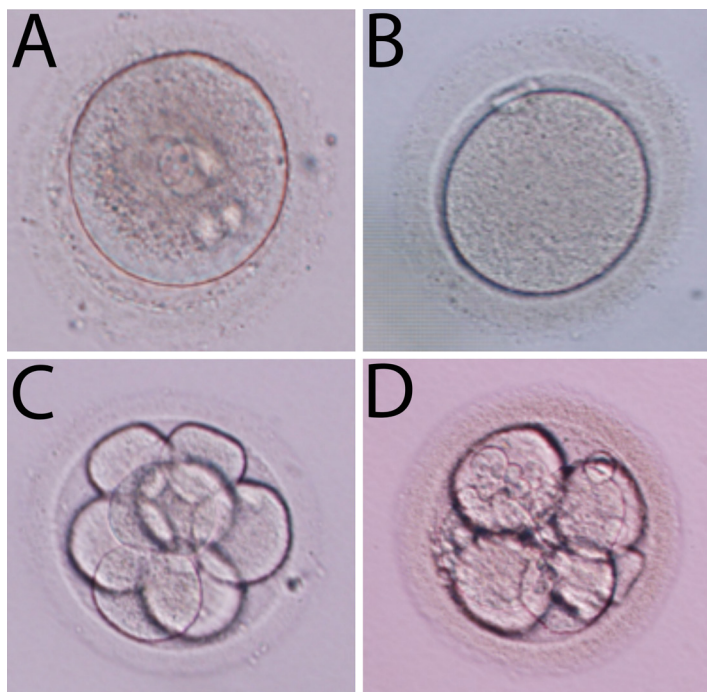


Figure 1. (A) Day 1 fertilized egg (2PN). (B) Day 1 unfertilized egg. (C) Day 3 good-quality embryo. (D) Day 3 low-quality embryo

The whole blood samples were collected in ethylenediamine tetraacetic acid containing tubes, and all samples were processed within 30 minutes after blood collection. The pretreatment hematological parameters of female and male patients were performed the third day of menstruation and before the spermiogram test with an automated hematology analyzer Beckman Coulter LH780 (USA). WBC (White Blood Cell), NE (Neutrophil), LY (Lymphocyte), MO (Monocyte), EO (Eosinophil), BA (Basophil), RBC (Red blood cell), HGB, MCV, MCH (Mean Cell Hemoglobin), RDW, PLT (Platelet) and PDW values were measured in the CBC.

Statistical Analysis

The data were analyzed by using SPSS statistical soft ware version 20.0 (SPSS Inc., Chicago, USA). Fertilization rate was calculated as a percentage of all fertilized oocytes. Embryo quality as embryo total score was calculated only in the day 3 embryos ($n = 50$) by multiplying embryo grade ($A = 3$, $B = 2$, $C = 1$) with the number of blastomeres for each embryo. Pearson's correlation coefficient was used to analyze the relationship between IVF results (fertilization rate, transplanted and good embryo quality rates) and hematological parameters. Point biserial correlation coefficient was used to analyze the relationship between clinical pregnancy rate and hematological parameters. Multiple linear regression analysis (stepwise model) was used to evaluate the relationship between hematological parameters and IVF results. Logistic regression analysis was used

to evaluate the relationship between hematological parameters and pregnancy rate. P value is less than 0.05 are considered statistically significant.

Results

Demographic data of the patient couples undergoing IVF treatment were shown in Table 1. Data were reported as mean±standard deviation or median (range).

Table 1. Demographic data of 50 couples undergoing IVF treatment

PARAMETER	
Female	
Age (years) ^a	30.7±5.2
Oocytes retrieved (n) ^a	9.2±4.6
M2 Oocytes (n) ^a	7.6±3.9
Oocytes fertilized (with 2PN) (n) ^a	5.4±3.4
Male	
Age (years) ^a	33.2±6.0
Semen volume (ml) ^a	3.3±1.2
Sperm concentration (106/ml) ^b	22 (0.01-90)
Progressive motility (%) ^a	44.9±20.3
4 points	149
Fertilization and laboratory parameters	
Fertilization rate (%) ^a	72.7±23.4
Transplantable Embryo rate (%) ^a	67.5±28.7
Good quality embryo rate (%) ^a	34.1±27
Values are (a), mean + standard deviation, and (b), median (range)	

According to statistical analyzes, while fertilization rate and PDW (rp = -0.341 *, p = 0.015) values was negative correlation, no correlation was found with other hematological parameters in male patients (p> 0.05). At the same time, there was a negative correlation between transplantable embryo rate and MCV value (rp = -0.320 *, p = 0.024), but no correlation was found between between transplantable embryo rate and other hematological parameters in male patients (p> 0.05). There was no correlation between male hematological parameters and both the good quality embryo rate and clinical pregnancy rate (p> 0.05).

In the female patients, fertilization rate possesses negative correlation with HGB value (rp = -0.332 *, p = 0.019) and positive correlation with RDW value (rp = 0.359 *, p = 0.010) in IVF treatment. At the same time, there was a negative correlation between the good embryo quality rate and PDW value (rp = -0.377 **, p = 0.007), and a negative correlation between the transplantable embryo rate and PDW value (rp = -0.305 *, p = 0.031). No correlation was observed between clinical pregnancy rate and female hematological parameters (p> 0.05).

There was no correlation between fertilization rate and both good quality embryo rate (p = 0.846) and transplantable embryo rate (p = 0.437). While clinical pregnancy rate was found positive correlation with both good embryo quality rate (rpb = 0.373**, p = 0.008) and

transplantable embryo rate (rpb = 0.292*, p = 0.039), no correlation was found with fertilization rate (p> 0.05).

Linear regression analysis stepwise model was used to test the relationship between hematological data and fertilization rate, transplantable embryo rate and good quality embryo rate in male and female patients receiving IVF treatment.

In the men, the fertilization rate possesses a negative relationship with the only PDW value (regression coefficient: -13.024, p = 0.015; Table 2), (corrected as -13.024, 0.015) no significant relationship was observed with other hematological data (p> 0.05). The linear regression equation for this relationship was: Fertilization Rate = 289.607-13.024 * PDW, and the estimated power obtained from the corrected r squared value is 9.8%.

Table 2. Regression of fertilization rate on male hematological data.

Independent variables	Regression coefficient	95% CI of Regression		t value	P value
		Lower bound	Upper bound		
Constant	289.607	115.977	463.236	3.354	0.002
PDW	-13.024	-23.450	-2.598	-2.512	0.015

The statistical analysis showed that only the MCV value has a negative negative relationship with the transplantable embryo rate (regression coefficient: -1.920, p = 0.024; Table 3). (corrected as -1.920)The linear regression equation for this relationship was: Transplantable embryo ratio = 237.695-1.920 * (corrected as 237.695-1.920)MCV and the estimated power obtained from the corrected r squared value is 8.3%. There was no significant relationship between good quality embryo rate and all hematological data in men (p> 0.05). A logistic regression analysis (enter model) was used to test the relationship between pregnancy rate and hematological data in male and female patients receiving IVF therapy. There was no statistically significant relationship between male hematological parameters and clinical pregnancy rate in two-way logistic regression analysis (p = 0.168).

Table 3. Regression of transplantable embryo rate on male hematological data

Independent variables	Regression coefficient	95% CI of Regression		t value	P value
		Lower bound	Upper bound		
Constant	237.695	90.773	384.657	3.252	0.002
MCW	-1.920	-3.573	-0.268	-2.336	0.024

In the statistical analysis in terms of associations of fertilization rate with hematological parameters in the female, only the RDW value produced a positive linear relationship with the fertilization rate (regression coefficient: 4.860 (corrected as 4.860), p = 0.012; Table 4), but there was no significant relationship between other hematological data and fertilization rate (p> 0.05). The linear regression equation for this relationship was: Fertilization Rate = 3.915 + 4.860 * RDW and the estimated power obtained from the adjusted r squared value is 10.9%.

Table 4. Regression of fertilization rate on female hematological data

Independent variables	Regression coefficient	95% CI of Regression		t value	P value
		Lower bound	Upper bound		
Constant	3.915	-49.360	57.190	0.148	0.883
RDW	4.860	1.124	8.597	2.617	0.012

The result showed that PDW was negative linear associated with both the transplantable embryo rate (regression coefficient: -16.855 (corrected as -16.855), $p = 0.033$; Table 5) and the good quality embryo rate (regression coefficient: -19.808 (corrected as -19.808), $p = 0.007$; Table 6) in IVF, but there was no significant relationship between other hematological data and both the transplantable embryo rate and the good quality embryo rate ($p > 0.05$). Linear regression equation for this relationship of PDW with the transplantable embryo rate: Transplantable embryo rate = $346.971 - 16.855$ (corrected as $346.971 - 16.855$) * PDW, and the estimated power obtained from the corrected r squared value was 7.3%. Linear regression equation for this association of PDW with the good quality embryo rate: Good quality embryo rate = $362.858 - 19.808$ (corrected as $362.858 - 19.808$) * PDW, and the estimated power obtained from the corrected r squared value is 12.5%. Logistic regression analysis also showed no statistically relationship between female hematological parameters and clinical pregnancy rate ($p=0.625$).

Table 5. Regression of transplantable embryo rate on female hematological data

Independent variables	Regression coefficient	95% CI of Regression		t value	P value
		Lower bound	Upper bound		
Constant	346.971	90.057	603.885	2.717	0.009
PDW	-16.855	-32.328	-1.382	-2.191	0.033

Table 6. RRegression of hematological values on good quality embryo rate in female patients

Independent variables	Regression coefficient	95% CI of Regression		t value	P value
		Lower bound	Upper bound		
Constant	346.971	90.057	603.885	2.717	0.009
PDW	-16.855	-32.328	-1.382	-2.191	0.033

Discussion

In the current study, we found that women patients' RDW value predicted a positive relationship in terms of fertilization rate and both female HGB and male PDW value possesses a negative association for fertilization rate. The results showed that the linear regression equation that reveals the relationship between male PDW and fertilization rate can be estimated by 9.8%,

and regression predictive power that indicates the relationship between female RDW and fertilization rate is 10.9 % or about one-tenth force. While these values significantly affected fertilization rates, but did not affect transplanted and good quality embryo rates. This situation can be explained as the fertilization rate is not related to embryo quality. According to our correlation analysis, there was no relationship between fertilization rate and embryo quality rate and it supports this situation. Similar to our results, Yoeli et al. [11] found in their study on 177 patients that increased fertilization rates obtained by intracytoplasmic sperm injection did not affect embryo quality. These results suggest that the fertilization process has no effect on embryo quality, but other cellular mechanisms are important on the embryo quality.

Platelets have increased activity in vascular problems, and secrete many of the mediators involved in hemostasis, inflammation, thrombosis, and atherosclerosis. Platelet indices, such as platelet and platelet volume, platelet distribution width and plateletcrit, are various parameters that reflect platelet activity [19]. Mahdavi-Zafarghandi et al. [20] showed that there was a relationship between high PDW levels in 50 varicocele patients and varicocele, which is a vascular disease and caused widespread male infertility. The findings in this study support the inverse proportion between male PDW values and fertilization rates in our study.

There are limited studies in the literature about the relationship between ovary and RDW value. In a study, high RDW levels were detected in women with polycystic ovary syndrome. RDW is used as part of the standard complete blood count and is a measure of the diversity of red blood cell volume [21]. Usually red blood cells are about 6-9 microns in diameter. However, some disorders cause a significant change in cell size [22]. Iron deficiency anemia, folate and vitamin B12 deficiency anemia usually have high RDW levels [23]. Many studies determined that oocytes have folate 2 receptors and follicle cells that support oocytes have folate 1 and 2 receptors and also folate, which is inversely correlated with high RDW levels affect oocytes and follicle cells [24]. This effect may explain the correct proportion between RDW levels and fertilization rate in women in our study.

In the current study, it was determined that both good quality embryo and transplantable embryo rates positively affect pregnancy rate. Many prospective and retrospective studies in the literature support this finding [25, 26]. No relation was found between hematological parameters of both sexes and clinical pregnancy rates. However, we found that some hematological data affect good quality embryo and transplantable embryo rates. Our study also showed that male MCV and female PDW value adversely affect transplantable embryo quality, and also female PDW value adversely affect good embryo quality. Although there was a relationship between good quality embryo rate and pregnancy rate, the fact that the relationship between the female PDW value and the pregnancy rate cannot be determined can be explained by the effect of this value has a small effect on the pregnancy rate. The linear regression equation' predictive power that reveals the relationship between MCV value and transplantable embryo rate is 8.3%, and between PDW value and transplantable and good quality embryo rates is 7.3% and 12.3%, respectively.

Platelets, key effector (corrected as Platelets, key effector...) cells for hemostatic and inflammatory responses, include alpha, dense granules, and lysosomal granules containing a large number of secretory products [27, 28]. Alpha granules are the most (50-60 %) (corrected as 50-60 %) and the greatest (200-400 nm) platelet granules and store a wide variety of proteins. As a result of a proteomic analysis, 284 different proteins were detected. The main ones are PF4, Ppbp b- thromboglobulin NAP-2, P-selektin, CD40L, TGF- β , PDGF, VWF, CD63, SDF-1, VEGF, BDNF, thrombospondins ve MMP-2. Dense granules are smaller (150 nm), fewer (3-8 percent) and store small molecules such as serotonin, glutamate, polyphosphate, ADP and histamine. Lysosomal granules are rare and contain glycohydrolase and degrading enzymes [29]. There are limited studies in the literature describing the relationship between platelets and ovaries. Murdoch found that the amount of thromboxane B2 (corrected as thromboxane B2) increased in the follicular fluid and follicle wall in study on preovulatory follicles [30]. LI et al. [31] detected that platelet activating factor (PAF) increased in ovulation and played a role in follicle rupture. Bodis et al. [32] showed that histamine increased estradiol secretion in the culture of granulosa cells obtained from follicular fluid from 17 female patients. Kreiner et al. [33] found that estradiol in human follicular fluid increases the quality of preovulatory oocytes. Koppan et al. [34] found in their cell culture study that serotonin increased estradiol secretions of gonadotropin-induced granulosa cells. Bodis et al. [35] determined that increased platelet aggregation and PAF amount were associated with excessive release of serotonin from the granulosa cells in the corpus hemarogicum formed after ovulation. It has been reported in many studies that BDNF stimulates folliculogenesis, oocyte maturation and early embryonic development [36]. In the light of the above information, it shows that platelets can play an important role in the paracrine control of folliculogenesis and ovulation due to the serotonin, histamine and BDNF stores in the ovary. There is no study in the literature explaining the relationship between PDW value and oocyte maturation, follicle development and ovarian physiology. The mechanisms we mentioned above may play a role in the inverse proportion between female PDW levels and fertilization rate in our study. Although female patients' PDW value especially strongly affect the embryo quality, the fact that this value does not affect the formation of pregnancy can be explained by the activation of many factors such as pregnancy-preventing molecular mechanisms in the maternal endometrium and maternal hormone levels.

Conclusion

As a result, we showed that some hematological parameters belonging to both men and women have effects on fertilization rate and embryo quality and these results adds new findings to the literature. In addition, the result that embryo quality increases clinical pregnancy rates, supports the findings of other studies in the literature. Our study indicated that especially the changes in female PDW values significantly affect embryo quality in women undergoing IVF and indirectly may affect pregnancy success negatively. The PDW values can be used to a predictive hematologic parameter for embryo quality and pregnancy success rate.

Conflict of interests

The authors declare that they have no conflict of interest

Financial Disclosure

All authors declare no financial support

Ethical approval

The necessary permission was obtained from the Ethics Committee of Ataturk University Faculty of Medicine (24.07.2014/8).

References

1. Cyna AM, Middleton P. Caudal epidural block versus other methods of postoperative pain relief for circumcision in boys. *Cochrane Database Syst Rev*. 2008;8:CD003005.
2. Prabhakaran S, Ljuhar D, Coleman R, et al. Circumcision in the paediatric patient: A review of indications, technique and complications. *J Paediatr Child Health*. 2018;54:1299-307.
3. Sir E, Eksert S, Zor M, et al. The analgesic efficacy of ultrasound guided unilateral transversus abdominis plane block in the pain management of shock wave lithotripsy. *Arch Esp Urol*. 2019;72:933-8.
4. Demiraran Y, Kocaman B, Akman RY. A Comparison of the postoperative analgesic efficacy of single dose epidural tramadol versus morphine in Children. *Br J Anaesth*. 2005;95:510-3.
5. Brenner L, Marhofer P, Kettner SC, et al. Ultrasound assessment of cranial spread during caudal blockade in children: The effect of different volumes of local anesthetics. *Br J Anaesth*. 2011;107:229-35.
6. Barash PG, Cullen BF, Stoelting RK. *Handbook of Clinical Anesthesia*. 6th edition. Lippincott Williams & Wilkins, Philadelphia, 2009.
7. Uguralp S, Mutus M, Koroglu A, et al. Regional anesthesia is a good alternative to general anesthesia in pediatric surgery: Experience in 1,554 children. *J Pediatr Surg*. 2002;37:610-3.
8. Şener M, Çalışkan E, Bozdoğan N, et al. Our experiences of paediatric caudal anaesthesia. *JARSS*. 2006;14:259-63.
9. Gezer E, Uğur B, Şen S, et al. Retrospective evaluation of caudal anaesthesia in pediatric surgery. *Meandros Med Dent J*. 2006;7:27-30.
10. Erdoğan NM, Kaymak S, Uğuz M, et al. Evaluation of analgesic effect of caudal anesthesia: A retrospective study. *J Contemp Med*. 2015;5:41-5.
11. Beyaz SG. Comparison of postoperative analgesic efficacy of caudal block versus dorsal penile nerve block with levobupivacaine for circumcision in children. *Korean J Pain*. 2011;24:31-5.
12. Irwin MG, Cheng W. Comparison of subcutaneous ring block of the penis with caudal epidural block for post-circumcision analgesia in children. *Anaesth Intensive Care*. 1996;24:365-7.
13. Weksler N, Atias I, Klein M, et al. Is penile block better than caudal epidural block for postcircumcision analgesia? *J Anesth*. 2005;19:36-9.
14. Bramwell RG, Bullen C, Radford P. Caudal block for postoperative analgesia in children. *Anaesthesia*. 1982;37:1024-8.
15. Walco GA, Cassidy RC, Schechter NL. Pain, hurt and harm. The ethics of pain control in infants and children. *N Engl J Med*. 1994;331:541-4.

16. Stang HJ, Snellman LW, Condon LM, et al. Beyond dorsal penile nerve block: a more humane circumcision. *Pediatrics*. 1997;100:E3.
17. Yeoman PM, Cooke R, Hain WR. Penile block for circumcision? A comparison with caudal blockade. *Anaesthesia*. 1983;38:862-6.
18. Kazak Bengisun Z, Ekmekci P, Haliloglu AH. Levobupivacaine for postoperative pain management in circumcision: caudal blocks or dorsal penile nerve block. *Agri*. 2012;24:180-6.
19. Locatelli B, Ingelmo P, Sonzogni V, et al. Randomized, double-blind, phase III, controlled trial comparing levobupivacaine 0.25%, ropivacaine 0.25% and bupivacaine 0.25% by the caudal route in children. *Br J Anaesth*. 2005;94:366-71.
20. Dobereiner EF, Cox RG, Ewen A, et al. Evidence-based clinical update: which local anesthetic drug for pediatric caudal block provides optimal efficacy with the fewest side effects? *Can J Anaesth*. 2010;57:1102-10.
21. Silvani P, Camporesi A, Agostino MR, et al. Caudal anesthesia in pediatrics: An update. *Minerva Anesthesiol*. 2006;72:453-9.
22. Jöhr M, Berger TM. Caudal blocks. *Paediatr Anaesth*. 2012;22:44-50.
23. Brown DL. *Regional Anesthesia and Analgesia*. 2nd edition. WB Saunders Company, Pennsylvania, 1996.
24. Akin M, Saydam S, Keskin G, et al. Caudal epidural anesthesia in pediatric urological surgery; a single institutional experience with 5536 cases. *Bozok Med J*. 2015;5:41-5.
25. Begeç Z, Durmuş M, Toprak Hİ, et al. Caudal block in children: A retrospective study of 2262 cases. *JTAICS*. 2005;33:288-94.
26. Ango PD, Kohou KL, Koné N, et al. Caudal anesthesia: experience in the post-operative analgesia in pediatric ambulatory surgery at the University Hospital of Treichville. *Open Journal of Anesthesiology*. 2017;7:400-6.
27. Ivani G, De Negri P, Lonngvist PA, et al. A comparison of three different concentrations of levobupivacaine for caudal block in children. *Anesth Analg*. 2003;97:368-71.
28. Ramboatiana R, Gassner C, Schoch JP, et al. [Postoperative analgesia for circumcision in children]. *Agressologie*. 1990;31:39-42.
29. Polat R, Akin M, Keskin G, et al. Comparison of dorsal penile block and caudal block with bupivacaine and oral ibuprofen in children undergoing circumcision. *J Anesth*. 2015;23:238-43.
30. Irsi C, Ar Yıldırım A, Ozdemir F. Caudal block with general anesthesia for outpatient pediatric surgery procedures. *J Anesth Crit Care Open Access*. 2017;7:00248.