Indomethacin Prior to Difficult Embryo Transfer is it a Solution?

Original Article

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ABSTRACT

Objectives: To find out the role of administration of indomethacin prior to embryo transfer in cases of difficult mock embryo transfer in in-*vitro* fertilization/ intra cytoplasmic sperm injection cycle in improving the reproductive outcomes.
Study Design: It is a randomized controlled trial.
Patients and Methods: The study was conducted in the in *vitro* fertilization units of the University hospital as well as a private unit from the 2nd of June 2018 till the 2nd of December 2018. A total of two hundred in *vitro* fertilization/ intra cytoplasmic sperm injection cycles who had difficult mock embryo transfer on the day of ovum pick up. Women were randomly assigned into two groups; group A (study group: n=100) will receive 100mg indomethacin rectal suppository 1-2 hours before embryo transfer, while group B (control group: n=100) did not receive any medications before the embryo transfer.
Results: Both groups were comparable regarding age, body mass index, basal hormones, and cause of infertility. The implantation (23.7% vs 20.8%, P value 0.906), clinical pregnancy (48% vs 40%, P value 0.254), and ongoing pregnancy (40% vs 36%, P value 0.560) rates were higher in the indomethacin group, but not reaching statistical significance.
Conclusion: Indomethacin , as adjuvant therapy, has no statistically significant role on in cases with difficult embryo transfer in in-*vitro* fertilization/ intra cytoplasmic sperm injection cycles.

Key Words: Difficult embryo transfer, implantation rate, indomethacin, IVF/ICSI cycles, pregnancy rate.

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INTRODUCTION

IVF treatment is a globally known method that gives couples the opportunity to form a family that they previously thought impossible. It is estimated that about 3.5 million people cannot get pregnant in the UK.[1]

With the great advances in assisted reproductive techniques (ART) and the careful precautions at the time of embryo transfer (ET) regarding the type of ET catheter, site of embryo deposition, elimination of cervical mucous, slow catheter extraction from the uterine cavity, choosing high quality embryos and the use of ultrasound during ET; still the success rate is 35-45%[2]. Personalization of the embryo transfer by transvaginal ultrasound and use a maleable catheter adapted accordingly was tried to overcome difficult embryo transfers due to anatomical causes[3].

A successful ET; in terms of implantation rate (IR) and pregnancy rate (PR), has been described as a way to deposit the newly formed embryos into the optimal location of the uterus without causing significant trauma. However, some women due to anatomical or physiological reasons present with a difficult ET.[4] These cases were associated with increased uterine contractions that affect the endometrial receptivity, implantation, and embryo rejection.[5]

Prostaglandins are lipid mediators which are formed from arachidonic acid by cyclooxygenase enzyme (COX). They have a role in endometrial preparation, ovulation, and implantation by increasing endometrial blood flow. However, prostaglandin excess at the time of ET has a deleterious effect[6,7].

Non-steroidal anti-inflammatory drugs (NSAIDs) block COX and hence prevent prostaglandin synthesis[8]. In animal studies, the administration of NSAIDs before or at the time of ET improved the pregnancy rates[9]. However there is no sufficient evidence to with or against analgesics to improve human ET outcomes[10].

Indomethacin is a strong NSAID which is classified during pregnancy as class C (FDA classification); it causes suppression of uterine contractions, has a strong anti-inflammatory effect, whilst not affecting

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the initial inflammatory reactions that are important for implantation[11]. It is not reported that indomethacin causes any birth defects, preterm labor, or low birth weight[12].

The aim of this study is to assess the role of indomethacin administration before ET on the reproductive outcome of IVF/ICSI cycles in cases with difficult mock embryo transfer (MET).

MATERIAL AND METHODS

It is a randomized controlled non-blinded, study which was held in the university hospital IVF center and private IVF center from the 2nd of June 2018 to the 2nd of December 2018, on 200 women undergoing fresh embryo transfer and fulfilling the inclusion criteria.

The study was approved by our ethical committee of the department and a written consent was obtained from all patients who were recruited.

Infertile patients undergoing IVF cycle with difficult MET on the day of ovum pickup, between 20-38 years of age, with early follicular Follicle stimulating hormone (FSH) level ≤10 IU/L, tubal, male infertility or unexplained causes of infertility undergoing fresh ET were included. While, those with history of repeated IVF failure, allergy to NSAID, bronchial asthma, peptic ulcer or inflammatory bowel disease, easy MET and those who refused to participate in the study were excluded.

They were randomly assigned into two groups according to computer generated random cards (Quickcalcs [Graphpad, La Jolla, CA, USA]) with a random block size of four; group A (study group n=100) and group B (control group n=100). The flow chart of the study is shown in (Figure 1).

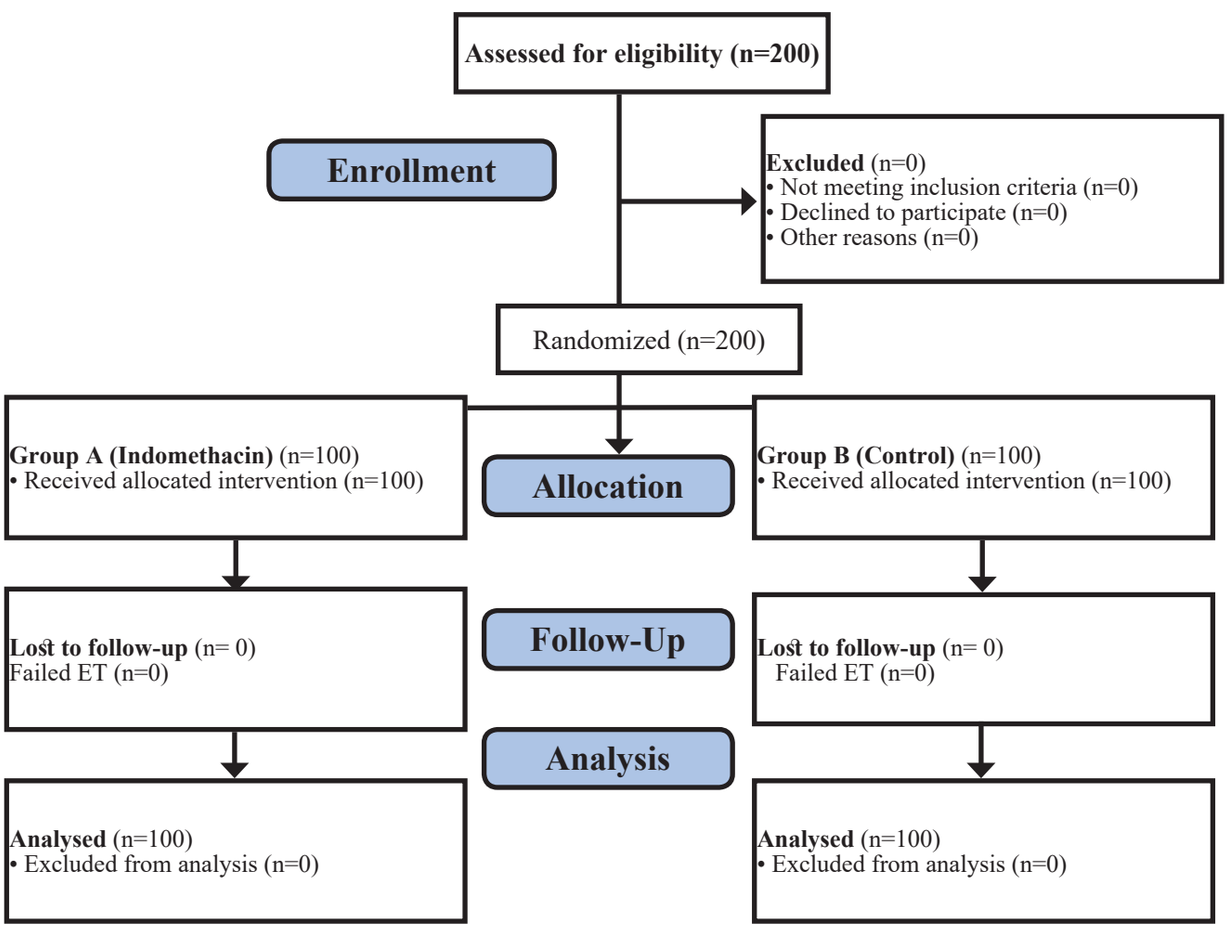

Fig. 1: The flow chart of the study

All patient underwent the long down regulation protocol; which was achieved by triptorelin 0.1 mg (decapeptyl 0.1 mg, Ferring, Malmo, Sweden) SC injection daily from day 21 of the previous cycle till the day of human chorionic gonadotrophin (HCG) administration.

On day 2 of the stimulation cycle down regulation was confirmed by checking serum E2<50 pg/ml, endometrial thickness < 5mm and quiescent ovaries. Stimulation was then initiated using Human Menopausal gonadotrophin (HMG) (Menogon, Ferring pharmaceuticals, Germany) in a dose of 150-225 IU daily as IM injection chosen according to the patient age, antral follicle count and ovarian reserve.

Serial trans-vaginal ultrasound assessment of follicular growth and serum E2 levels were done, starting from day 6 of the cycle and onward, with adjustments of gonadotropin dose and monitoring frequency according to patient response.

Once 3 or more leading follicles reached ≥18mm, Human chorionic gonadotrophin (hCG) 10000 IU IM (Choriomon; IBSA, Switzerland) was given. Trans-vaginal ultrasound guided egg collection was done 34-36 hours after hCG trigger under general anesthesia. MET was done after finishing ovum pick up to assess the different variables as uterine cavity measurements and uterine direction.

Women with difficult MET according to Tomas *et al*. 2002 criteria[13] were assigned randomly into two groups according to computer generated random cards; group A; study group (n=100): received indomethacin 100 mg rectal suppository (Kahira Pharma&Chem, Ind, CO. Cairo-Egypt) 1-2 hours before ET, group B; control group (n=100): did not receive any medications before ET.

Transfer of 2 embryos was done 2–3 days after ovum pick up, according to the number and quality of available embryos, under ultrasound guidance using labotec catheter (Labotec, Gottingen Germany).

For luteal phase support; progesterone vaginal pessaries (Cyclogest, Alpharma, UK) 400 mg twice daily was used from the day of ovum pick up till the day of the pregnancy test and continued till 12th week of gestation if pregnancy is documented.

The primary outcome was the implantation rate. While, the secondary outcomes of the study included the clinical and ongoing pregnancy rates. To ensure the homogeneity of the two groups the E2 levels on day of HCG administration, endometrial thickness, number of oocytes retrieved, number of MII oocytes, number of fertilized oocytes, number of transferred embryos, Mucous and blood in ET catheter were recorded.

Implantation rate is defined as the number of total gestational sacs divided by the total number of embryos transferred.

Clinical pregnancy rate is defined as positive β-HCG with the presence of one or more gestational sac detected by ultrasound after 4 weeks from ET.

Ongoing pregnancy rate is defined as the presence of viable pregnancy at 12-week gestation.

Sample size calculation was done using the comparison of clinical pregnancy rate between cases doing embryo transfer with and without indomethacin pre-treatment. Calculation was done based on comparing 2 proportions from independent samples in a prospective study using Chi test, the α-error level was fixed at 0.05, the power was set at 80% and the intervention groups (case: control) ratio was set at 1. As previously published by Moon *et al*., 2004, the incidence of clinical pregnancy in indomethacin pre-treatment was 46.6% while it was 27.6% in non-treated women[12]. Accordingly, the minimum optimum sample size should be 100 participants in each arm. Sample size calculation was done using PS Power and Sample Size Calculations software, version 3.0.11 for MS Windows (William D. Dupont and Walton D. Vanderbilt, USA).

Data was statistically described in terms of mean ± standard deviation (± SD) or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student t test for independent samples in comparing normally distributed data and Mann Whitney U test for independent samples for comparing not normally distributed data. For comparing categorical data, Chi-square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5. P values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows.

RESULTS

The study was conducted on 200 infertile women undergoing IVF/ICSI cycles with difficult MET encountered on the day of ovum pick up.

Both groups were comparable regarding age, BMI, basal hormones and cause of infertility which is shown in (Table 1).

Table 1: Basal characteristics of both groups

Variables	Group A (Indomethacin group n = 100)	Group B (No indomethacin group n = 100)	*P value*
Age (years)	27.8 ± 3.5	27.3 ± 3.1	0.571
BMI (kg/m2)	25.7 ± 3.6	24.6 ± 2.6	0.292
Basal FSH (IU/L	6.3 ± 1.5	5.8 ± 1.3	0.262
Basal LH (IU/L)	5.4 ± 1.8	4.3 ± 1.3	0.061
Basal Estradiol (pg/ml)	47.7 ± 11.2	45.3 ± 8.6	0.491
Cause of infertility (n/n, %)			
Unexplained	8/100, 8%	12/100, 12%	0.345
Tubal	10/100, 10%	14/100, 14%	0.384
Male	82/100, 82%	74/100, 74%	0.172

**P value* < 0.05 is considered statistically significant, all values presented as mean and standard deviation, unless stated otherwise

Regarding the cycle characteristics of both groups there were no statistical significant difference between them regarding E2 levels on day of HCG administration, endometrial thickness, number of oocytes retrieved, number of MII oocytes, number of fertilized oocytes, number of transferred embryos, Mucous and blood in ET catheter (Table 2).

Table 2: Cycle characteristics of both groups

Variables	Group A (Indomethacin group n = 100)	Group B (No indomethacin group n = 100)	*P value*
E2 levels on HCG day (pg/mL)	2155 ± 1346	2008 ± 888	0.766
Endometrial thickness (mm)	11.3 ± 1.2	11.1 ± 1.4	0.469
Number of collected oocytes	8.4 ± 5.1	9.3 ± 4.3	0.415
Number of MII oocytes	6.5 ± 3.5	6.7 ± 3.4	0.870
Number of fertilized oocytes	4.6 ± 2.6	5.2 ± 2.4	0.311
Number of transferred embryos	2.9 ± 0.6	2.8 ± 0.4	0.638
Mucous in embryo transfer catheter (n/n,%)	16/100, 16%	12/100, 12%	0.414
Blood in embryo transfer catheter (n/n, %)	4/100, 4%	8/100, 8%	0.233

**P value* < 0.05 is considered statistically significant, all values presented as mean and standard deviation, unless stated otherwise

There was also no significant statistical difference between the two groups regarding implantation rate, clinical and ongoing pregnancy rates (Table 3).

Table 3: Reproductive outcomes of both groups

Variables	Group A (Indomethacin group n = 100)	Group B (No indomethacin group n = 100)	*P value*
No of sacs (mean ± SD)	0.7 ± 0.9	0.5 ± 0.6	0.642
Implantation rate (%)	23.7 %	20.8 %	0.906
Clinical pregnancy rate (%)	48/100 (48%)	40/100 (40%)	0.254
Ongoing pregnancy rate (%)	40 /100(40%)	36/100 (36%)	0.560

**P value* < 0.05 is considered statistically significant

DISCUSSION

In this randomized open label study, we assessed the impact of indomethacin in patients undergoing IVF/ICSI with difficult MET aiming to improve their reproductive outcomes. What was challenging and new in this study, was recruiting patients with difficult MET whom expectedly may have more manipulations during their ET and thus increasing the inflammatory reaction and uterine contractions that may impair implantation. Our data showed a slight improvement in the reproductive outcomes in the study group, but unfortunately not reaching statistical significance.

Uterine contractions are one of the most fundamental components of the uterine receptivity. Studies have shown a six-fold increase in uterine contractions in IVF/ICSI cycles when measured before ET as compared to the natural cycle before ovulation[14].

A systematic review byArora *et al*. examined the most successful method resulting in highest pregnancy rates (PRs) in patients with difficult ET. They found that the majority of the difficult ETs were caused by cervical stenosis and the most common treatment was cervical dilation. Hegar dilators used a minimum of 3 weeks before ET showed to have higher PR.[15].

Many drugs have been potentially tested to improve IVF success rates. Treatment strategies included the use of oxytocin antagonist, Atosiban was tested in many studies and showed promising effects on implantation, especially in cases with recurrent implantation failure[16-19]. More recently, Nolasiban is currently being tested in many clinical trials with encouraging potential to decrease contractions and improve uterine blood flow hence enhancing the receptivity of the endometrium to embryo implantation[20].

Many NSAIDs have also been used in IVF-ET procedures, namely indomethacin and piroxicam. In this study we used indomethacin as it concurs two possible

mechanisms of action decreasing the inflammatory response in the endometrium that result from both mechanical manipulation and introduction of foreign particles, and in the myometrium by reducing its activity[21].

Some animal studies have shown a favorable effect from using NSAIDs in mice and cows after ET on the implantation and pregnancy rates[9,22] Sohrabvand *et al*., in 2009, performed a pilot study on 66 infertile women using indomethacin rectal suppositories before ET and reported an improvement in pregnancy rates. But later in 2014 the same group of researchers studied the effect of piroxicam before ET and declared no significant benefit on pregnancy rates[23].

Another two studies used piroxicam before ET and showed a favorable effect on the implantation and pregnancy rates[12,24]. While, Dal Prato *et al*., contradicted their findings by using piroxicam prior to ET and found no change in implantation and pregnancy rates[25].

Bernabeu *et al*., 2006 studied the effect of indomethacin on implantation rate in donor oocyte recipients to minimize the effect of possible confounders related to oocyte quality, and they concluded that indomethacin did not increase implantation rate in their study group. Finally, Kumbasar *et al*., studied both indomethacin and piroxicam among three groups of patients and concluded that NSAIDs has no additional effect on reproductive outcomes in IVF/ICSI cycles[26,27].

The strength of this study lies in its randomization, as well as recruiting cases with difficult ET. Limitations of this study include its non blinded nature to both the physician and patients, patient compliance was not recorded, and only one dose of indomethacin was used prior to ET.

CONCLUSION

In conclusion the use of a single dose of indomethacin before ET in cases of difficult ET did not improve implantation rate, clinical and ongoing pregnancy rates.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. NHS. Infertility. 2012. http://www.nhs.uk/conditions/Infertility/Pages/Introduction.aspx

2. Schild RL, Knobloch C, Dorn C, Fimmers R, Van Der Ven H, Hansmann M. Endometrial receptivity in an in *vitro* fertilization program as assessed by spiral artery blood flow, endometrial thickness, endometrial volume, and uterine artery blood flow. Fertility and Sterility. 2001 Feb 1;75(2):361-6. https://www.sciencedirect.com/science/article/pii/S0015028200016952

3. Larue L, Bernard L, Moulin J, Massari A, Cassuto NG, Bouret D, et.al. Evaluation of a strategy for difficult embryo transfers from a prospective series of 2,046 transfers. F&S Reports. 2021 Mar 1;2(1):43-9.

4. Kasapoglu I, Aslan K, Orhan A, Albayrak O, Haskoylu S, Kuspinar G, Avcı B, Uncu G. The Effect of Embryo Transfer Process Duration on Implantation Success. Gynecology Obstetrics & Reproductive Medicine. 2022:1-6.

5. Singh N, Gupta P, Mittal S, Malhotra N. Correlation of technical difficulty during embryo transfer with rate of clinical pregnancy. Journal of human reproductive sciences. 2012 Sep;5(3):258. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3604832/

6. Fanchin R, Ayoubi JM, Righini C, Olivennes F, Schönauer LM, Frydman R. Uterine contractility decreases at the time of blastocyst transfers. Human Reproduction. 2001 Jun 1;16(6):1115-9. https://academic.oup.com/humrep/article/16/6/1115/619500

7. Chakraborty I, Das SK, Wang J, Dey SK. Developmental expression of the cyclo-oxygenase-1 and cyclo-oxygenase-2 genes in the peri-implantation mouse uterus and their differential regulation by the blastocyst and ovarian steroids. Journal of molecular endocrinology. 1996 Apr 1;16(2):107-22. https://jme.bioscientifica.com/view/journals/jme/16/2/jme_16_2_002.xml

8. Maslow KD, Lyons EA. Effect of prostaglandin and antiprostaglandin on midcycle myometrial contractions. Fertility and sterility. 2004 Aug 1;82(2):511-3. https://www.sciencedirect.com/science/article/pii/S0015028204008635

9. Dawood MY. Nonsteroidal antiinflammatory drugs and reproduction. American journal of obstetrics and gynecology. 1993 Nov 1;169(5):1255-65. https://www.sciencedirect.com/science/article/abs/pii/000293789390292Q

10. Scenna FN, Hockett ME, Towns TM, Saxton AM, Rohrbach NR, Wehrman ME, et.al. Influence of a prostaglandin synthesis inhibitor administered at embryo transfer on pregnancy rates of recipient cows. Prostaglandins & other lipid mediators. 2005 Dec 1;78(1-4):38-45. https://www.sciencedirect.com/science/article/abs/pii/S1098882305000353

11. Practice Committee of the American Society for Reproductive Medicine. Performing the embryo transfer: a guideline. Fertility and sterility. 2017 Apr 1;107(4):882-96.

12. Gupta U, Malhotra N, Varma SK, Chaudhury RR. Effect of intrauterine administration of antiprostaglandin drugs on implantation in the rat. Contraception. 1981 Sep 1;24(3):283-8. https://www.sciencedirect.com/science/article/abs/pii/001078248190041X

13. Moon HS, Park SH, Lee JO, Kim KS, Joo BS. Treatment with piroxicam before embryo transfer increases the pregnancy rate after in *vitro* fertilization and embryo transfer. Fertility and sterility. 2004 Oct 1;82(4):816-20. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Moon+HS%2C+Park+SH%2C+Lee+JO%2C+Kim+KS%2C+Joo+BS.2004%3B+Treatment+with+piroxicam+before+embryo+transfer+increases+the+pregnancy+rate+after+in+*vitro*+fertilization+and+embryo+transfer.+Fertil+Steril.+82%284%29%3A816-820.&btnG=

14. Tomás C, Tikkinen K, Tuomivaara L, Tapanainen JS, Martikainen H. The degree of difficulty of embryo transfer is an independent factor for predicting pregnancy. Human Reproduction. 2002 Oct 1;17(10):2632-5. https://academic.oup.com/humrep/article/17/10/2632/607745

15. Ayoubi JM, Epiney M, Brioschi PA, Fanchin R, Chardonnens D, de Ziegler D. Comparison of changes in uterine contraction frequency after ovulation in the menstrual cycle and in in *vitro* fertilization cycles. Fertility and sterility. 2003 May 1;79(5):1101-5 https://www.sciencedirect.com/science/article/pii/S0015028203001791

16. Arora P, Mishra V. Difficult embryo transfer: A systematic review. Journal of human reproductive sciences. 2018 Jul;11(3):229. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6262663/

17. Mishra V, Agarwal H, Goel S, Roy P, Choudhary S, Lamba S. A prospective case–control trial to evaluate and compare the efficacy and safety of atosiban versus placebo in In *vitro* Fertilization-embryo transfer program. Journal of human reproductive sciences. 2018 Apr;11(2):155. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6094537/

18. Li J, Chen Y, Wang A, Zhang H. A meta-analysis of atosiban supplementation among patients undergoing assisted reproduction. Archives of gynecology and obstetrics. 2017 Oct 1;296(4):623-34. https://link.springer.com/article/10.1007/s00404-017-4455-0

19. Huang QY, Rong MH, Lan AH, Lin XM, Lin XG, He RQ, et.al. The impact of atosiban on pregnancy outcomes in women undergoing in *vitro* fertilization-embryo transfer: A meta-analysis. PloS one. 2017 Apr 19;12(4):e0175501. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0175501

20. Kim SK, Han E, Kim SM, Lee JR, Jee BC, Suh CS, et.al. Efficacy of oxytocin antagonist infusion in improving in *vitro* fertilization outcomes on the day of embryo transfer: A meta-analysis. Clinical and experimental reproductive medicine. 2016 Dec 1;43(4):233-9. https://ecerm.org/journal/view.php?doi=10.5653/cerm.2016.43.4.233

21. Visnova H, Tournaye HJ, Humberstone A, Terrill P, Macgregor L, Loumaye E. A placebo-controlled, randomized, double-blind, phase 3 study assessing ongoing pregnancy rates after single oral administration of a novel oxytocin receptor antagonist, nolasiban, prior to single embryo transfer. Fertility and Sterility. 2018 Sep 1;110(4):e45. https://www.fertstert.org/article/S0015-0282(18)30733-7/abstract

22. Bernabeu R, Roca M, Torres A, Ten J. Indomethacin effect on implantation rates in oocyte recipients. Human Reproduction. 2005 Nov 10;21(2):364-9 https://academic.oup.com/humrep/article/21/2/364/614112

23. Schlapp G, Goyeneche L, Fernández G, Menchaca A, Crispo M. Administration of the nonsteroidal anti-inflammatory drug tolfenamic acid at embryo transfer improves maintenance of pregnancy and embryo survival in recipient mice. Journal of assisted reproduction and genetics. 2015 Feb 1;32(2):271-5. https://link.springer.com/article/10.1007/s10815-014-0378-x

24. Sohrabvand F, Haghollahi F, Maasomi M, Shariat M. Effect of piroxicam on ART outcome: a pilot study. International journal of fertility & sterility. 2014 Oct;8(3):243. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4221509/

25. Firouzabadi RD, Ghandi S, Tayebi N. Effect of administration of single dose piroxicam before embryo transfer on implantation and pregnancy rates in IVF cycles. Journal of Biological Sciences. 2007 Jan 1;7(1):123-6. https://scialert.net/fulltextmobile/?doi=jbs.2007.123.126

26. Dal Prato L, Borini A. Effect of piroxicam administration before embryo transfer on IVF outcome: a randomized controlled trial. Reproductive biomedicine online. 2009 Oct 1;19(4):604-9. https://www.sciencedirect.com/science/article/abs/pii/S1472648309000236

27. Kumbasar S, Gül Ö, Şık A. Evaluation of the effect of indomethacin and piroxicam administration before embryo transfer on pregnancy rate. Journal of Obstetrics and Gynaecology Research. 2017 Mar;43(3):536-42. https://obgyn.onlinelibrary.wiley.com/doi/abs/10.1111/jog.13244