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Research Article

Relationship Between Late Follicular Phase Progesterone Level and the Risk of Ectopic Pregnancy Following IVF-Fresh Embryo Transfer Cycles: A Case-Control Study

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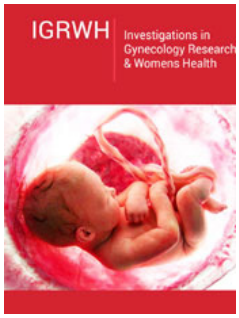

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Abstract

Objective: To assess whether increased progesterone level during controlled ovarian stimulation increases the risk of Ectopic Pregnancy (EP) following fresh Embryo Transfer (ET).

Design: Retrospective case-control study.

Materials and Methods: All cases (n=29) of EP (study group) were compared to 79 cases of documented viable intra-uterine pregnancies (control group) between August 2009 and December 2016 at an academically-affiliated fertility center (Clinique Ovo, Montreal, Canada). The control group cases were selected based on a random number generator model on a year-to-year basis. Bivariate analysis was conducted to assess the effect of all collected variables on EP.

Result: The two groups did not differ significantly in factors traditionally associated with EP (previous EP, endometriosis, tubal disease, history of pelvic infection, and abdominal surgery). Patients with EP were more likely to have had day 3 rather than a day 5 transfer (P=0.001), had double rather than a single ET (*P*=0.001), and finally were more likely to have had a difficult transfer (P=0.004), independently of the use of a rigid catheter. Median progesterone level measured on the day before or on the day of ovulation trigger was not statistically different between the two groups (2.55nmol/L for the study group vs. 2.52nmol/L for the control group, *P*= 0.169).

Conclusion: No relationship between late follicular phase progesterone level and EP could be demonstrated in this study. This can be due to a neutralizing effect of the opposing physiologic actions of progesterone: promoting uterine quiescence that favors intrauterine implantation on one side, and decreasing tubal ciliary beat frequency that promotes tubal implantation on the other.

Keywords: Ectopic pregnancy; Assisted reproduction technology; Progesterone level; IVF; Tubal physiology

Introduction

Ever since the first pregnancy achieved with *In Vitro* Fertilization (IVF) and Embryo Transfer (ET) was ectopic [1], the association between Assisted Reproductive Technology (ART) and this morbid complication has been very solid, with some reports claiming that the risk of Ectopic Pregnancy (EP) may be increased as much as 2-fold in women who conceive via ART [2,3]. It remains very difficult to pinpoint the mechanisms responsible for this association, but some suggested explanations include inadvertent direct tubal ET or its natural migration

from the uterus into the tube. In a mock intrauterine ET with 50µl of radiopaque fluid, Knutzen et al. [4] showed that the material was transferred into the tube in almost half of the cohort, which is a lot higher than the natural incidence of EP. Accordingly, for the embryo to implant in the tube, there should be some pathological mechanism which prevents its movement back into the uterine cavity [5]. Independently of tubal damage that might be the reason for infertility and the need for ART to start with, the hormonal milieu may play a key role in the pathogenesis of EP.

In IVF-ET cycles, Progesterone (P) concentrations are supraphysiologic and exceed these of normal conception due to the multifollicular growth, the presence of multiple corpus lutea, and the additional iatrogenic supplementation for luteal phase support [6]. On one side, high P level contributes to uterine quiescence [7]. This decreased uterine contractility favors embryonic implantation in the uterine cavity as opposed to its migration into the fallopian tubes [6]. On the other side, high P level reduces ciliary beat frequency in both humans and mice fallopian tubes [8,9]. This resultant ciliary dysfunction could prevent the migration of the embryo back from the tubes into the uterus.

The evidence of P-related increase in EP comes from contraceptives' studies. The risk of EP among women using progestin oral pills or progestin implants is 2 to 5-fold compared to other women of childbearing age [10,11]. The incidence of EP with progesterone-bearing IUD's is also considerably greater (16.3% of the pregnancies) than observed with placebo device (5.1% of the pregnancies) [12]. However, to the best of our knowledge, no study to date assessed whether similar P-mediated increase in the risk of EP is seen in IVF-ET cycles. Hence the objective of our study is to assess whether increased late follicular phase P level during controlled ovarian stimulation increases the risk of EP following fresh ET.

Materials and Methods

Study design

This is a retrospective case-control study where all cases (n=29) of EP (study group) were compared to 79 cases of documented viable intrauterine pregnancies (control group) which were selected based on a random number generator model on a year-to-year basis. All pregnancies were the result of an IVF cycle followed by fresh ET conducted between August 2009 and December 2016 at Clinique Ovo, a private fertility center affiliated with the Université de Montréal, Canada.

The main exposure was progesterone level measured in nmol/L on the day before or on the day of ovulation trigger. Other exposures included known EP risk factors (history of pelvic inflammatory disease, endometriosis, documented tubal disease, previous EP and major abdominal surgery), and the IVF-ET cycle characteristics (type of protocol used, blood estradiol level prior to trigger, number of ovules obtained, endometrial thickness, day of ET, number of embryos transferred, difficulty of transfer, and the use of rigid catheter). Demographics as in age and smoking status were also accounted for.

ART protocols & assessment of primary exposure

Protocols were divided between antagonist, long agonist and short flare-up depending on the physician's personalized decision for every case. Stimulation was achieved either with recombinant or urinary gonadotropins. A GnRH agonist or a GnRH antagonist was used for down-regulation. To monitor ovarian stimulation, serum levels of estradiol and P were recorded, followed by transvaginal ultrasound. Serum P was dosed by the ARCHITECT Progesterone (Abbott, *i*2000SR), a Chemiluminescent Microparticle Immunoassay (CMIA).

Thirty-six hours after administration of urinary hCG, oocyte retrieval was performed under IV sedation. To fertilize the oocytes, either standard IVF or ICSI were used based on fertility indication. Single or double fresh ET was undertaken on day 3 (cleavage stage) or day 5 (blastocyst stage) under trans-abdominal ultrasound guidance, with the embryos transferred around 1.5cm from the fundal endometrial surface. Note that ultrasounds, oocyte retrievals, and ET's were performed by REI subspecialists or REI fellows according to the standardized protocols of the clinic. Luteal support was provided with 50 mg of intramuscular P daily and transdermal estradiol patches releasing 100mcg daily.

Ethical considerations

The study was approved by our Institutional Review Board. Because of its anonymous chart review nature, and absence of any medical intervention, no patient consent was needed. There were no known conflicts of interest or financial support for this work that could have influenced its outcome.

Statistical analysis

Quantitative variables were reported as percentage or median with 25th & 75th percentiles, as appropriate. SPSS v22.0 (IBM, Armonk, New York) was used to carry the bivariate analysis to evaluate the association of each collected variable with EP. A *P*-value < 0.05 was considered statistically significant.

Result

As outlined in Table 1, there was no statistically significant difference in patients' age between the study group and the control group, with a median of 36 and 34 years old respectively (*P*= 0.257). Bivariate analysis showed that patients with EP were significantly more likely to be suppressed with a GnRH agonist (41.4%), as opposed to 86.1% of those with intrauterine pregnancies being suppressed with a GnRH antagonist (*P*= 0.002). Nevertheless, this difference in stimulation protocols did not lead to any significant difference in the number of retrieved oocytes (11 vs. 10, *P*= 0.377), endometrial thickness at ovulation trigger (10.0 vs. 10.4 mm, *P*= 0.116), nor serum P level (2.55 vs. 2.52nmol/L, *P*= 0.169) between the study group and control group respectively. There was a trend for higher blood estradiol level in patients with EP, but it did not reach statistical significance (7668 vs. 5862pmol/L, *P*= 0.060).

Table 1: Results of the bivariate analysis. IUP: intra-uterine pregnancies; PID: pelvic inflammatory disease.

	EP (n=29)	IUP (n=79)	
	n (%)	n (%)	P value
History of PID	0 (0)	2 (2.5)	1.000
Endometriosis	0 (0)	5 (6.3)	0.321
Previous EP	2 (6.9)	1 (1.3)	0.175
Tubal disease	2 (6.9)	8 (10.1)	1.000
Major abdominal surgery	1 (3.4)	0 (0)	0.269
Difficult transfer	4 (13.8)	0 (0)	0.004
Use of rigid catheter	2 (6.9)	0 (0)	0.07
Smoking	0 (0)	11 (13.9)	0.034
Stimulation protocol			0.002
Antagonist	17 (58.6)	68 (86.1)	
Long	7 (24.1)	3 (3.8)	
Short	5 (17.2)	8 (10.1)	
	Median (25^{th}-75^{th} centile)		P value
Age (years)	36 (31-38)	34 (31-37)	0.257
Endometrial thickness (mm)	10.0 (8.8-11.4)	10.4 (9.2-12.8)	0.116
Number of retrieved oocytes	11 (8-15)	10 (6-14)	0.377
Day of transfer	3 (3-3)	3 (3-5)	0.001
Number of embryos transferred	1 (1-2)	1 (1-1)	0.001
Progesterone (nmol/L)	2.55 (2.15-4.14)	2.52 (2.00-2.98)	0.169
Estradiol (pmol/L)	7668 (541012800)	5862 (4637-7928)	0.06

No patient with EP had a history of pelvic inflammatory disease nor endometriosis, suggested by symptomatology or by direct surgical visualization. Only 2 out of the 29 (6.9%) had previous confirmed EP and a resultant tubal pathology seen either surgically, or on hysterosalpingography or Sono hysterography. Only 1 patient in the ectopic group (3.4%) had a previous open myomectomy, while no patient in the control group had any major abdominal surgery. None of these abovementioned variables reached statistical significance between the two groups.

It was noted that smoking habits were more frequent among carriers of normal pregnancies, with 13.9% of them reporting tobacco consumption, as opposed to none of those with EP (P= 0.034). Patients with EP were more likely to have had a cleavage-stage rather than a blastocyst transfer (P= 0.001), had double rather than a single ET (P= 0.001), and finally were more likely to have had a difficult transfer (13.8% vs. 0%, P= 0.004), independently of the use of a rigid catheter (6.9% vs. 0%, P= 0.070). 4 out of the 29 ET's leading to EP were judged difficult by the performing physician, and 50% of them (2 out of 4) required the use of a rigid catheter in order to negotiate the cervical canal.

Discussion

The fact that P level did not significantly differ between patients with either ectopic or intrauterine pregnancies might be the result of some neutralizing effect between its two opposite hormonal actions: decreasing uterine contractility on the one hand, and altering tubal peristalsis on the other. With estradiol tending to be higher in the ectopic group, we thought that its role in tubal physiology and resultant ectopic implantation outweighs that of Wu Z et al. [13] showed in a large retrospective multicenter study that the risk of EP was higher in women with both elevated P and estradiol on the day of hCG trigger (18.10%), when compared to those with isolated elevated P (9.5%, P< 0.05), but not different from the group with isolated elevated estradiol (11.67%, NS) [13]. This is supported by some existing evidence from bovine models where estradiol was shown to affect oviductal smooth muscle contractility [14]. Human studies failed to completely reproduce this finding, with even some reports showing the complete opposite: treatment with clomiphene citrate- an estrogen receptor antagonist at the level of the fallopian tube- increased the frequency of tubal implantation [15].

Regarding the association between the type of stimulation protocol and the risk of EP, our findings oppose those of Rombauts et al. [16] who found that antagonist cycles were associated with a 2-fold increased risk compared with agonist one [16]. Agonist suppression is associated with an early elevation of serum P compared to suppression with an antagonist, which is commonly referred to as "premature luteinization" in the literature [17,18]. Accordingly, we postulate that it is the cumulative effect of the early rise in P throughout stimulation that could impact tubal physiology, rather than its absolute value just prior to ovulation.

Overall, the risk for EP increases approximately 2-fold among women who smoke [19,20]. The suggested responsible mechanism

may involve a lower efficiency of oocyte-cumulus complex capture or a decreased tubal ciliary beat frequency directly induced by chemical components of cigarette smoke [21,22]. Nevertheless, an extrapolation from spontaneous pregnancies to those resulting from ovarian stimulation might be misleading, specifically that smoking can affect the level of bioavailable P by altering the corticosteroid-binding globulins [23-25] resulting in such unexpected outcomes whereby 13.9% of patients with normal pregnancies were smokers as opposed to none with EP.

It has been proposed that decreased uterine contractility later in the luteal phase and the larger diameter of the blastocyst would interfere with its ability to reflux through the ostium, protecting against tubal implantation [6,26], which was consistent with our results of significantly less blastocyst transfer in the study group compared to the control group. However, higher implantation potential per embryo at the blastocyst stage than cleavage stage may negate these effects, with a rich literature to argue in that direction [26-28].

When it comes to the number of embryos transferred, it's logical to assume that the associated risk of EP follows a direct positive correlation. In a population-based cohort analysis of 44,102 pregnancies from the Australian and New Zealand registries, pregnancies following single ET had a 1.2% ectopic rate, significantly lower than double ET (1.8%, $P< 0.01$) [29]. Once again, our findings are in line with the available medical evidence.

Even though strong evidence links a difficult ET to poor outcomes like reduced clinical pregnancy and live birth rates [30,31], Listijono et al. [30] found no significant difference in EP rates based on transfer difficulty [30]. This contradicts our results, whereby 13.8% of the ectopic cases had a challenging ET. Accordingly, more studies are needed to evaluate whether increased irritation and resultant uterine contractility, coupled with endometrial trauma, decreases endometrial receptivity and flushes the embryo into the tubes, making it a more hospitable environment for implantation.

One of the major strengths of this study lies in the fact that well-proven classical risk factors for EP were similar between the two groups, and these include: pelvic inflammatory disease, endometriosis, previous ectopic, tubal disease, and history of major abdominal surgery. These comparable demographics eliminate the statistical uncertainty when trying to control for variables; thus, any significant relationship can be solely attributed to the assessed exposure itself, independently of potential confounders. Even endometrial thickness, a relatively less solid risk factor for EP, was also comparable between the two groups. This was based on the theory that a thin endometrium prior to transfer is associated with an upward direction of the uterine peristalsis that might favor movement of the embryo into the tubes [16].

The study is further strengthened by being limited to a single-center which eliminates inter-laboratory variability in dosing serum hormone levels. We recognize that this study has some limitations, mainly a retrospective design and a limited sample size. But with EP being a rare outcome, a prospective approach with a larger sample size would be an illogical attempt to reach the desired statistical power. The fact that ET's were performed by different physicians could have confounded the findings, but the standardized protocol within the center in terms of transfer technique, volume of transferred material, and transfer depth helps to minimize such concerns. In addition, this heterogeneity allows a better extrapolation into daily clinical practice where physicians with different backgrounds and experiences perform the procedure. Finally, because as of January 2011 a freeze-all policy has been applied at our center for cycles with highly elevated P on the day of trigger, it makes it more difficult to answer our original study question whether an elevated P increases the risk of EP following fresh ET, since such cycles are automatically excluded from the analysis.

Conclusion

To the best of our knowledge, this is the first study to assess the relationship between late follicular phase P level and the risk of EP in the setting of ART. Even though no correlation could be found between the two, a hormonal etiology of EP cannot be firmly ruled out because of intermingling variables. These variables include the role of estradiol, external and lifestyle factors that might affect the concentration of the bioavailable P, and the exact timing within the cycle when P starts to rise which might be dependent on the type of stimulation protocol used. This will set the ground for future work whereby the estradiol/P ratio can be followed progressively throughout the period of controlled ovulation stimulation to better evaluate any association with EP.

References

1. Steptoe PC, Edwards RG (1976) Reimplantation of a human embryo with subsequent tubal pregnancy. Lancet 1(7965): 880- 882.
2. Marcus SF, Brinsden PR (1995) Analysis of the incidence and risk factors associated with ectopic pregnancy following *in-vitro* fertilization and embryo transfer. Hum Reprod 10(1): 199-203.
3. Braude P, Rowell P (2003) Assisted conception. III--problems with assisted conception. BMJ 327(7420): 920-923.
4. Knutzen V, Stratton CJ, Sher G, McNamee PI, Huang TT, et al. (1992) Mock embryo transfer in early luteal phase, the cycle before in vitro fertilization and embryo transfer: A descriptive study. Fertil Steril 57(1): 156-162.
5. Pyrgiotis E, Sultan KM, Neal GS, Liu HC, Grifo JA, et al. (1994) Ectopic pregnancies after *in vitro* fertilization and embryo transfer. Journal of Assisted Reproduction and Genetics 11(2): 79-84.
6. Chang HJ, Suh CS (2010) Ectopic pregnancy after assisted reproductive technology: What are the risk factors? Curr Opin Obstet Gynecol 22(3): 202-207.
7. Fanchin R, Righini C, Ziegler D, Olivennes F, Ledee N, et al. (2001) Effects of vaginal progesterone administration on uterine contractility at the time of embryo transfer. Fertil Steril 75(6): 1136-1140.
8. Paltieli Y, Eibschitz I, Ziskind G, Ohel G, Silbermann M, et al. (2000) High progesterone levels and ciliary dysfunction--a possible cause of ectopic pregnancy. J Assist Reprod Genet 17(2): 103-106.
9. Bylander A, Nutu M, Wellander R, Goksor M, Billig H, et al. (2010) Rapid effects of progesterone on ciliary beat frequency in the mouse fallopian tube. Reprod Biol Endocrinol 8: 48.

10. Beral V (1975) An epidemiological study of recent trends in ectopic pregnancy. Br J Obstet Gynaecol 82(10): 775-782.

11. Liukko P, Erkkola R, Laakso L (1977) Ectopic pregnancies during use of low-dose progestogens for oral contraception. Contraception 16(6): 575-580.

12. Tatum HJ, Schmidt FH (1977) Contraceptive and sterilization practices and extrauterine pregnancy: A realistic perspective. Fertil Steril 28(4): 407-421.

13. Wu Z, Li R, Ma Y, Deng B, Zhang X, et al. (2012) Effect of HCG-day serum progesterone and oestradiol concentrations on pregnancy outcomes in GnRH agonist cycles. Reprod Biomed Online 24(5): 511-520.

14. Huang N, Liu B, Dong Z, Mao W, Zhang N, et al. (2015) Prostanoid receptors EP2, EP4, and FP are regulated by estradiol in bovine oviductal smooth muscle. Prostaglandins Other Lipid Mediat 121(Pt B): 170-175.

15. Shao R, Feng Y, Zou S, Weijdegard B, Wu G, et al. (2012) The role of estrogen in the pathophysiology of tubal ectopic pregnancy. Am J Transl Res 4(3): 269-278.

16. Rombauts L, McMaster R, Motteram C, Fernando S (2015) Risk of ectopic pregnancy is linked to endometrial thickness in a retrospective cohort study of 8120 assisted reproduction technology cycles. Hum Reprod 30(12): 2846-2852.

17. Edelstein MC, Seltman HJ, Cox BJ, Robinson SM, Shaw RA, et al. (1990) Progesterone levels on the day of human chorionic gonadotropin administration in cycles with gonadotropin-releasing hormone agonist suppression are not predictive of pregnancy outcome. Fertil Steril 54(5): 853-857.

18. Silverberg KM, Burns WN, Olive DL, Riehl RM, Schenken RS (1991) Serum progesterone levels predict success of *in vitro* fertilization/embryo transfer in patients stimulated with leuprolide acetate and human menopausal gonadotropins. J Clin Endocrinol Metab 73(4): 797-803.

19. Saraiya M, Berg CJ, Kendrick JS, Strauss LT, Atrash HK, et al. (1998) Cigarette smoking as a risk factor for ectopic pregnancy. Am J Obstet Gynecol 178(3): 493-498.

20. Bouyer J, Coste J, Shojaei T, Pouly JL, Fernandez H, et al. (2003) Risk factors for ectopic pregnancy: A comprehensive analysis based on a large case-control, population-based study in France. Am J Epidemiol 157(3): 185-194.

21. Knoll M, Talbot P (1998) Cigarette smoke inhibits oocyte cumulus complex pick-up by the oviduct *in vitro* independent of ciliary beat frequency. Reprod Toxicol 12(1): 57-68.

22. Riveles K, Iv M, Arey J, Talbot P (2003) Pyridines in cigarette smoke inhibit hamster oviductal functioning in picomolar doses. Reprod Toxicol 17(2): 191-202.

23. Schafer HH, Villiers JD, Sivukhina E, Lewis J, Wande D, et al. (2013) Altered homeostasis of systemic glucocorticoids as related to obesity, glucose tolerance and smoking. Horm Metab Res 45(3): 245-251.

24. Field AE, Colditz GA, Willett WC, Longcope C, McKinlay JB (1994) The relation of smoking, age, relative weight, and dietary intake to serum adrenal steroids, sex hormones, and sex hormone-binding globulin in middle-aged men. J Clin Endocrinol Metab 79(5): 1310-1316.

25. Saladin ME, McClure EA, Baker NL, Carpenter MJ, Ramakrishnan V, et al. (2015) Increasing progesterone levels are associated with smoking abstinence among free-cycling women smokers who receive brief pharmacotherapy. Nicotine Tob Res 17(4): 398-406.

26. Keegan DA, Morelli SS, Noyes N, Flisser ED, Berkeley AS, et al. (2007) Low ectopic pregnancy rates after *in vitro* fertilization: Do practice habits matter? Fertil Steril 88(3): 734-736.

27. Milki AA, Jun SH (2003) Ectopic pregnancy rates with day 3 versus day 5 embryo transfer: A retrospective analysis. BMC Pregnancy Childbirth 3(1): 7.

28. Rosman ER, Keegan DA, Krey L, Liu M, Licciardi F, et al. (2009) Ectopic pregnancy rates after *in vitro* fertilization: A look at the donor egg population. Fertil Steril 92(5): 1791-1793.

29. Li Z, Sullivan EA, Chapman M, Farquhar C, Wang YA (2015) Risk of ectopic pregnancy lowest with transfer of single frozen blastocyst. Hum Reprod 30(9): 2048-2054.

30. Listijono DR, Boylan T, Cooke S, Kilani S, Chapman MG (2013) An analysis of the impact of embryo transfer difficulty on live birth rates, using a standardised grading system. Hum Fertil (Camb) 16(3): 211-214.

31. Phillips JA, Martins WP, Nastri CO, Raine-Fenning NJ (2013) Difficult embryo transfers or blood on catheter and assisted reproductive outcomes: a systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol 168(2): 121-128.

