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Comparison the Effect of Oxytocin and Human Chorionic Gonadotropin on Ovulation

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To clarify the possible role of oxytocin (OT) on ovulation and conception compared to Human Chorionic Gonadotropin (HCG), a randomized controlled trial was carried out. One hundred clomiphene citrate-resistant anovulatory women, who were referred from April 2004 to September 2006, to the gynecologic clinics of teaching hospitals in Tabriz, Iran, were randomly allocated into 2 equal groups to take either Clomiphene Citrate (CC) plus OT (5 IU) or CC plus HCG (10, 000 IU). The size and the number of follicles by vaginal ultrasonography determined the administration of HCG or OT. The serum progesterone concentration was measured to prove the ovulation. The ovulation and the pregnancy rate of the two groups were compared. Sixty-seven women completed the study. There was no significant differences between the two groups in the rate of ovulation (92% vs. 84%, respectively, $p>0.05$). No significant differences were noticed between the two groups in the mean number of follicles at the first month ($p = 0.63$), but at the second month, the OT group had significantly more follicles ($p = 0.024$). The rate of pregnancy was similar (16.4 vs. 18.3 at two consecutive cycles). The OT group had more pain than the HCG group ($p<0.0005$) and the HCG group had more hyperstimulation rate than the OT group ($p<0.05$). The findings of this study provided essential insights to the physiological roles of OT in human follicular development and oocyte maturation. Oxytocin administration in CC-resistant patients gave a unique opportunity to use alternatives to stimulate oocyte maturation, in comparison with HCG and was associated with a fewer side effects.

Key words: Oxytocin, human chorionic gonadotropin, clomiphene citrate-resistant, ovulation

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INTRODUCTION

Ovulation disorders is among the most common causes of female infertility (30-40%) (Rossin, 2006). To date, various studies have been conducted to evaluate the effectiveness of any treatment strategy for the induction of ovulation and many medical approaches have been developed (Nandanwar *et al*., 1999; Laven *et al*., 2002; Shalevl *et al*., 1995; Johnson, 2002; George *et al*., 2003; Sayyah Melli *et al*., 2005).

A trend was observed toward the hyperstimulation and assisted reproduction as a first-line treatment in the anovulatory infertility, especially in patients with the polycystic ovarian syndrom (PCOS). This shift in clinical practice is not based on the sound scientific evidence (Jerome *et al*., 2004). In fact, even in the IVF programs, emphasis may now be directed toward the development of simple, minimal stimulation protocols (Hohmann *et al*., 2003; Macklon and Fauser, 2003; Karande *et al*., 1999). Clomiphene citrate is a choice and will successfully to induce ovulation in the women without having the other co-existing infertility factors (Hughes *et al*., 2000; Dickey and Holtkamp, 1996).

It is estimated that approximately 10% of patients are refractory to this protocol (Imani *et al*., 1998; Yildiz *et al*., 2003; Survey *et al*., 2000).

Several strategies have been suggested to induce ovulation in the CC-resistant anovulatory women (George *et al*., 2003; Braian and Estes, 2003; Malkawi *et al*., 2002; Keay and Jenkins, 2003; Mitwally and Casper, 2001), but there is no evidence-based algorithm to guide the initial and subsequent choices of the ovulation inducing methods in these patients (Dyer, 2002). Direct ovarian stimulation with HCG or Ovidrel is an obvious combination and was used to stimulate follicle maturation and rupture (Breck, 2007), but it is, by no means, the only option that merits consideration (Agarwal and Buyalos, 1999; Zreik *et al*., 1999). There is no spontaneous LH surge in these patients (Willis *et al*., 1998; Ievy *et al*., 2000). HCG is similar to luteinizing hormone (LH) and is used in ovulation induction as a substitute for LH to trigger ovulation (Sanjay *et al*., 1995). The timing of the spontaneous LH surge is always optional and related to the estrogen levels, but that of HCG treatment is not (Taylor *et al*., 1997; Marco, 1999). HCG must be administrated at an appropriate time. The patterns of follicle development may to be abnormal in patients with PCOS, who received CC (Yong *et al*., 1992; Taylor *et al*., 1997; Almahbobi *et al*., 1996; Hartitha *et al*., 2003) and the time when HCG is to be administrated may be inappropriate. That is why some patients could not achieve pregnancy or get problems such as ovarian hyperstimulation syndrom (OHSS). If HCG is administrated untimely, it may likely induce atresia than ovulation, or may lead to premature oocyte maturation (Opsahl *et al*., 1996).

Very little data is available in the literature addressing the occurrence and clinical relevance of this phenomenon (Opsahl *et al*., 1996; Martinez *et al*., 1991; Speroff and Fritz, 2005). It is nice to find another treatment strategy, because many couples are unable or reluctant to pursue HCG treatment once fully advised of associated costs, logistical demands and risks (Collins, 2001).

Simulation of a physiologic environment which mimics the endogenic hormone stimulation, a method which works naturally without the mentioned side effects, may solve this problem.

According to the investigations, OT has a possible role in the process of ovulation (Gimpl and Fahrenholz, 2001; Furuya *et al*., 1995; Copland *et al*., 2002; Mass *et al*., 1992). Amico *et al*. (1981) showed that the rise in the level of OT and Estrogen-Stimulated Neurophysin (ESN) chronologically correlates with a rise in the level of estrogen in the midcycle. The rise in estrogen neurophysin begins 10 h after the rise in estrogen and precedes that of the LH surge (Speroff and Fritz, 2005). Since OT chronologically correlates with a rise in the level of E at midcycle, a role for OT in ovulation may be considered.

However, several recent primate studies have added to our understanding of the role of OT on ovulation (Einspanier *et al*., 1995; Hrabovszky *et al*., 1998; Einspanier *et al*., 1997). The role of OT in the regulation, the timing and initiation of ovulation is controversial (Furuya *et al*., 1995; Copland *et al*., 2002).

Oxytocin is a neuropeptid and mainly produced in two hypothalamic nuclei, where from is secreted into the CSF, directly to the circulation, portal system and anterior pituitary and influence gonadotropin secretion (McEwen, 2004).

Furthermore, OT-like materials are found in the ovary, in follicular fluid, the oviduct, the testis and the adrenal glands, suggesting that this neurohypophyseal peptide has roles as a paracrine or autocrine hormone (Mass *et al*., 1992). In addition, these authors showed that OT has stimulatory effects on estradiol (E2) and progesterone (P) secretion. Therefore, OT-induced E2 release may be responsible for the increased P release.

As observed in other studies, because the ovulation is a major problem in most infertile women with CC resistance, finding a successful method which works naturally without the mentioned side effects, prompted us to design a randomized controlled trial to investigate the objectives of this study and to clarify the possible role of

OT on ovulation, pregnancy and ovarian hyperstimulation syndrom (OHSS) in CC anovulatory women and compare the results with HCG.

MATERIALS AND METHODS

One hundred infertile women with anovulation, who met the criteria of the study thoroughly, were selected among the patients who were referred to the gynecologic clinics of Tabriz University of Medical Sciences, East Azarbayejan Province, North West of Iran, from April 2004 to September 2006. Patients who had chronic anovulation and classical PCOS (Breck, 2007) with CC resistance (Speroff and Fritz, 2005), were under 40 years old, had patent tubes on hysterosalpingography and no other pelvic pathology were included in the study. The patients who were over 40 years of age, had hypersensivity to any oxytocic medications, history of cardiovascular disease and history of taking anti-hypertensive medications were excluded. In addition, women with blood pressure less than 90/60 mmHg, pelvic inflammatory disease, uterine abnormalities and abnormal physical exam, abnormal spermogram, ovarian cysts, anxiety, excess prolactin levels, thyroid diseases and other causes of anovulation were excluded. Participants were randomly allocated into 2 equal groups to receive either 10.000 IU HCG (Choriomon; manufactured by IBSA Institute Biochimique SA, CH-6903 Lugano), or 5 IU Oxytocin (OXYTIP; manufactured by IPDIC, Rasht, Iran) intramuscularly after CC stimulation (100 mg day[Hi]$^{-1}$ from day 3 to day 9) had induced enlarged ovarian follicles (>18 mm in diameter). Decision to give a 5 IU dose of OT was made according to the pilot study. The patients who had larger follicles (more than 30 mm in diameter) were withdrawn. The patients who achieved pregnancy at first or second month were also withdrawn. All participants were evaluated for the levels of plasma progesterone one week after the injection of OT, or HCG. A follow-up visit was arranged for both groups every month at the expected time of menstruation until 2 months after recruitment or at any time during the trial course if pregnancy was achieved.

The study was approved by the Tabriz University of Medical Sciences Research Committee. The Medical Ethics Committee of Tabriz University of Medical Sciences approved the research study. All participants were given adequate information and consent was obtained from each participant. Rating scales were used to score the levels of pain from 0 to 3 (0 representing no pain and 3 representing severe pain).

Descriptive statistics for the variables of interest were calculated. The measured values were given as median (range) or means (SD). Comparison of the categorical variables was made using chi-square (χ^2) tests where appropriate. Independent t-test was used for comparison of continuous variables between two groups. For all statistical analysis, p-value less than 0.05 was considered significant. The statistical analysis was performed using SPSS 14.0/$_{win.}$

RESULTS

Among 100 infertile women who assigned randomly to treatment, 50 received CC + HCG and 50 received CC + OT. Of these patients, 85 women followed the trial and 67 women (39 and 28, respectively) completed it. The flow of the patients in the study is presented in Fig. 1.

Some characteristics of the patients are summarized in Table 1. The results of t-test to compare means for the independent groups showed that the difference between two treatment groups with regard to the size of the follicles in the first month was significant (t = 2.06, df = 98, p = 0.042) and the OT group had more dominant follicles. In the second month, the difference between two treatment groups with regard to the size of the follicles were not significant (t = 1.49, df = 76, p = 0.140). In addition, no significant differences was shown in the number of follicles in the first month (t = 0.483, df = 77.45, p = 0.630), but there were significant difference between the groups in the number of follicles in the second month (t = 2.29, df = 76, p<0.024) (Table 2). The results of the same test showed that the difference between plasma progesterone levels in the first month were not significant (t = 1.67, df = 93, p = 0.097), but in the second month were statistically significant (t = 3.48, df = 69, p = 0.001). The mean level of progesterone in the second month in the OT group was higher (Table 2). There was significant difference between groups at the level of the pain in the first and the second months and the OT group had more pain than the HCG group (χ^2 = 14.64, df = 1, p<0.0005 and χ^2 = 17.52, df = 1, p<0.0005, respectively). Only mild ovarian hyperstimulation occurred in the first and second

Table 1: Basic characteristics of both groups

Variables	HCG group (mean±SD)	OT group (mean±SD)	t	df	p-value*
Age (year)	24.79±4.07 (18-36)	25.70±5.19 (19-38)	0.922	86.99	0.359
Gravida	0.20±0.53 (0.0-3)	0.66±1.27 (0.0-7)	2.040	65.64	0.045
Parity	0.12±0.32 (0-1)	16.0±0.46 (0-2)	0.495	98.00	0.622
Abortion	0.08±0.34 (0-2)	0.34±1.09 (0-7)	1.590	58.31	0.116

*: Differences were considered statistically significant at p<0.05

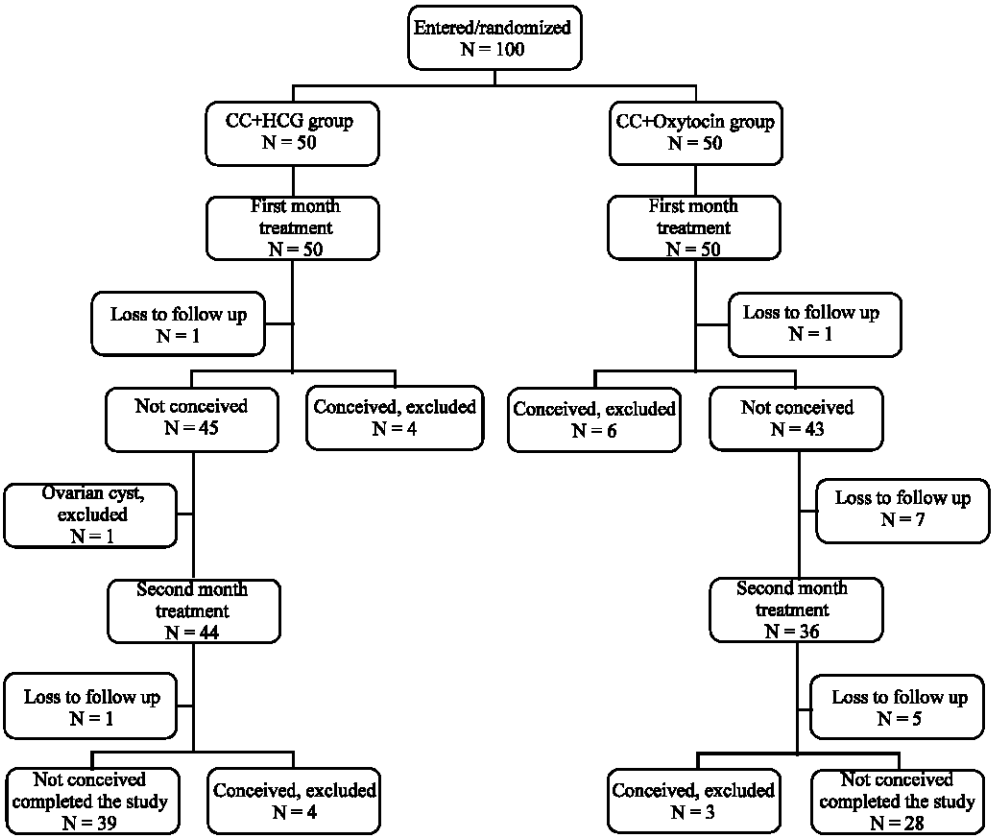

Fig. 1: Flow of the patients

Table 2: Results of treatment for 100 women administrated HCG (n = 50) and OT (n = 50), in two months

Variables	HCG group (Mean ± SD)	OT group (Mean±SD)	t	df	p-value*
Size of follicles at the first month (mm)					
Length	18.53±5.96	19.69±5.32	2.06	98.00	0.042
Width	17.52±5.37	20.40±6.37			
Size of follicles at the second month (mm)					
Length	15.30±6.84	18.41±4.70	1.49	76.00	0.140
Width	18.89±3.40	19.11±5.50			
No. of follicles at the first month	2.57±2.47	2.38±1.39	0.48	77.45	0.630
No. of follicles at the second month	2.12±1.94	3.25±2.41	2.29	76.00	0.024
Plasma progestrone level of first month	8.71±5.96	10.61±5.29	1.67	93.00	0.097
Plasma progestrone level of second month	7.96±5.42	12.79±6.13	3.48	69.00	0.001

*: Differences were considered statistically significant at $p<0.05$

Table 3: Results of treatment for 100 women administrated HCG (n = 50) and OT (n = 50), in two month

Results	HCG group	OT group	χ^2	df	p-value*
Levels of pain at the first month (%)					
No pain	87.2	51.0	14.640	1	<0.0005
Mild	12.8	49.0			
Moderate	-	-			
Severe	-	-			
Levels of pain at the second month (%)					
No pain	88.4	35.3	17.520	1	<0.0005
Mild	4.7	64.7			
Moderate	7.0	-			
Severe	-	-			
Hyperstimulation (first month) (%)					
No	67.0	91.3	0.449	1	0.503
Mild	13.0	8.7			
Moderate	-	-			
Severe	-	-			

Table 3: Continued

Results	HCG group	OT group	χ^2	df	p-value*
Hyperstimulation (second month) (%)					
No	85.7	92.70	0.613	1	0.592
Mild	14.3	7.20			
Moderate	-	-			
Severe	-	-			
Pregnancy rate (%)					
First month	8.2	12.20	0.555	2	0.758
Second month	8.2	6.10			

*: Differences were considered statistically significant at $p<0.05$

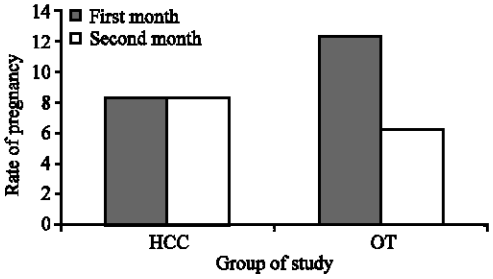

Fig. 2: The rate of pregnancy in group 1 and 2 at the first and second month

months in each group, which was not statistically significant (χ^2 = 0.449, df = 1, p = 0.503 and χ^2 = 0.613, df = 1, p = 0.592, respectively). In the OT group ovarian hyperstimulation rate was lower (Table 3). The rate of pregnancy in group 1 in the first and second months was 16.4% (4 and 4, respectively) and in the second group was 18.3% (6 and 3, respectively) (Fig. 2).

DISCUSSION

Recent advances in endocrinology pave the way for the clinical application of the hormonal therapy in the treatment of infertile women. The induction of ovulation in anovulatory infertile women who are resistant to CC treatment is a profound clinical challenge (Survey *et al*., 2000). A number of studies have examined the effect of various protocols on the ovulation and pregnancy rates in CC-resistant infertile women and several combinations have been proposed for the induction of an endogenous LH surge and the final stages of oocyte maturation in these patients (Bayram *et al*., 2001; Nandanwar *et al*., 1999; Shalevl *et al*., 1995; Anonymous, 2001; Takumi, 2004; Fauser *et al*., 2002). Many new treatment modalities have been introduced over the years without proper evaluation for the efficacy and safety (Johnson *et al*., 2003; Dyer, 2002; Collins, 2001; Daya, 2000).

Human chorionic gonadothropin is among the several protocols that has commonly been used in combination with CC in these patients as a substitute for LH for many years (Shalevl *et al*., 1995; Johnson, 2002; Agarwal and Buyalos, 1995; Zreik *et al*., 1999; Sanjay *et al*., 1995; Martinez *et al*., 1991). This approach has been considered to be the standard of care for the induction of final stages of oocyte maturation (Hoff *et al*., 1983). HCG has high cost and needs close monitoring. These are the two important facts that come in the way of their widespread use. On the other hand, HCG is also believed to contribute to an increased risk for the OHSS (Delvigne and Rosenberg, 2002).

A number of studies have examined the effect of various protocols on ovulation. In the study of Shalevl *et al*. (1995), a low dose of gonadotropin-releasing hormone agonist (GnRHa), has been used in place of HCG to induce ovulation in CC-resistant patients. In another study, Takumi (2004), showed that a low-dose, short-term metformin, combined with CC, improved the ovulation rates in CC-resistant infertile women with PCOS.

Other combinations such as GnRH antagonists and recombinant LH and HCG are not the first choice and cost benefit and should be kept for the patients who undergo IVF (Fauser *et al*., 2002; Anonymous, 2001).

Therefore, alternative regimens for this problem are being explored. The regimens could increase the pregnancy and ovulation rate and reduce the side effects would be the convenience of therapy. In this study, the researchers compared the results of a traditional therapy with a new treatment strategy (OT), for the final maturation of oocyte and hence LH surge. According to the literature, up to now, no study has examined the OT in infertility programs. This study appears to be the first to show the use of OT in the infertility treatment protocols. Although, the findings are reported here for the first time, the baseline findings are in line with the previous researches that suggest a role for OT in ovulation (Gimpl and Fahrenholz, 2001; McEwen, 2004; Furuya *et al*., 1995; Copland *et al*., 2002; Amico *et al*., 1981; Mass *et al*., 1992). The results showed that this intervention mimics physiologic circumstances in anovulatory women; hence, inducing ovulation. An overall 34.7% of patients (16.4 vs. 18.3), responded to clomiphene citrate plus HCG or OT by pregnancy in two

consecutive cycles and 84 vs. 92% for HCG and OT for ovulation, respectively. In addition, no serious side effect was observed in the OT group.

Several investigations were conducted to clarify the role of OT in the central and peripheral tissues, e.g., uterus, placenta, amnion, corpus luteum, testis and heart (Gimpl and Fahrenholz, 2001; Speroff and Fritz, 2005). Oxytocin which is found in preovulatory follicles and the corpus luteum, may have direct actions on the pituitary, ovary, uterus and fallopian tubes during ovulation (Furuya *et al*., 1995; Copland *et al*., 2002; Mass *et al*., 1992).

In addition, previous studies have shown that the OT and OT-like hormones facilitate reproduction in all vertebrates at several levels (Gimpl and Fahrenholz, 2001; Furuya *et al*., 1995).

Akerlund (2004) found that the uterus itself may be a source of OT mRNA in the endometrium of non-pregnant women with the highest levels around the time of ovulation.

Oxytocin is also, found in follicular fluid and circulation. In a study, Copland *et al*. (2002) showed that OT significantly augmented the effects of agents that stimulate the HCG-AMP pathway by enhancing the uptake of cholesterol and inhibition of progesterone metabolism. The results of their study showed that OT alone does not stimulate progesterone synthesis, but might be involved in fine-tuning progesterone release. Other studies have also shown that OT acts as a luteotropic hormone (Gimpl and Fahrenholz, 2001; McEwen, 2004; Einspanier *et al*., 1995; Einspanier *et al*., 1997). In the report of Maas *et al*. (1992), intraluteal application of OT in young corpus luteum resulted in a net stimulation of progesterone. Although OT and its receptor have been identified in human ovary, its regulatory role in granulosa cell or corpus luteum function has not been clearly defined (Copland *et al*., 2002).

Furuya *et al*. (1995) have reported the existence of OT in mammalian granulosa-luteal cells after ovulation. They obtained cumulus cells with mature oocytes from experimental and clinical *in vitro* fertilization-embryo transfer (IVF-ET) programs. In addition, these authors detected OT gene expression in mouse and human cumulus cells. Furthermore, OT receptor gene expression was clearly demonstrated in human cumulus cells and a weak positive signal was observed in human oocytes. These results were the first observations of simultaneous OT and OTR gene expression in cumulus cells, suggesting that the ovarian OT might have some physiological role in the early stage of embryo development.

Another study has also shown a possible functional role for OT in human sexual response. Carmichael *et al*. (1994) showed a correlation with the levels of OT and orgasm intensity. Perhaps OT induces muscle contractions during orgasm. The release of OT is episodic (spurt). Ordinarily, there are about 3 spurts every 10 min (Speroff and Fritz, 2005). Oxytocin is released during coitus, probably by the Ferguson reflex (vaginal and cervical stimulation) but also by olfactory, visual and auditory pathways.

There is another substantial experimental evidence suggesting that OT is able to influence anterior pituitary hormones as a hypothalamic regulatory factor (Hrabovszky *et al*., 1998).

Because gonadotropin releasing hormone (GnRH) and OT are competing substrates for hypothalamic degradation enzymes, it has been hypothesized that OT in the portal blood at the midcycle can inhibit the metabolism of GnRH, thus increasing the amount of GnRH available (Speroff and Fritz, 2005).

Oxytocin may induce endogen LH rise and acts naturally. Except for the low-grade abdominal pain, the patients with OT experienced fewer side effects. In addition, OT did not induce hyperstimulation. Besides the improved safety profile, taking 5 IU OT is so much more simple and convenient and there is also the decreased cost of medication preparation. Moreover, this strategy may significantly reduce the chances for OHSS and could be suggested to as a alternative for HCG in IVF programs. As the study was not blinded and was open for the researchers, a bias could have occurred and influenced the study results. Ideally, further studies should be performed to gain more insight into the effectiveness of OT on ovulation.

CONCLUSIONS

In conclusion, the results showed that OT plus CC is as effective as HCG plus CC and could be suggested to use in place of HCG to induce ovulation in CC-resistant patients. Oxytocin mimics physiologic circumstances. These findings suggest a role for the OT on ovulation. Oxytocin may regulate ovulation, induce endogenic LH rise, induce the final stages of oocyte maturation, assist follicle rupture and maintain corpus luteum. On the whole, OT is considered better as a regulatory factor for reproduction and the OT system is more investigated for the mechanisms of actions. Oxytocin may hold promise as a novel treatment modality for the infertile women and could be kept in reserve for patients who are refractory to CC.

LIMITATIONS

- Small sample size.
- No other study to compare the results

- Sampling bias, because the study was not blinded and could influence the study results.
- The inability to control for extraneous factors such as: Psychological factors and previous prescriptions

FUTURE RESEARCHES

- Larger sample sizes with a more heterogeneous population should be conducted so that study results may be generalized and increase the validity of the study findings
- Additional studies that examine the association between
  - The time of OT administration and LH rise
  - Determine the different doses of OT for the rupture of follicles
  - Determine the level of OT in normal cycles before and after ovulation
  - Determine the level of OT in cycles that induced by CC alone, before and after ovulation
  - Determine the level of OT in cycles that induced by CC and OT before and after ovulation
  - Determine the level of OT in follicular fluid in IVF cycles and its relation to the plasma levels of OT, LH and progesterone.
  - Determine the level of LH after the administration of OT

COMMENTS

- Researches examining the relationships between OT administration, pregnancy rate, pregnancy outcome and OHSS.
- Further investigations comparing OT with other agents should also be explored

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