Introduction

Pre-term delivery occurs in 8-10% of all births and accounts for approximately 80% of early neonatal morbidity and death, extremely pre-term infants are at risk of cerebral palsy and at a later stage behavioural, fine motor and learning difficulties ***(Cetingoz et al., 2011).***

As a consequence, reducing pre-term birth is a primary goal in perinatal healthcare. Advances in medical treatment have allowed the prolongation of pregnancy in women at risk of early delivery. However, during the last two decades, the number of pregnancies at high risk for pre-term delivery has escalated as a result of the increase in multifetal pregnancies due to in-vitro fertilization programmes ***(Vasibuch et al., 2010).***

Pre-term and low birth weight deliveries are more common in singleton pregnancies conceived after ovulation induction and assisted reproductive technology (ART) than in singleton pregnancies conceived spontaneously ***(Locci et al., 2006).***

On the basis of these considerations, at risk pregnancies, such as those resulting from intra-cytoplasmic sperm injection (ICSI), should be treated to improve pregnancy outcome ***(Hassan et al., 2011).***

Short cervix during pregnancies (diagnosed by vaginal ultrasound) is predictive of pre-term birth among asymptomatic women, though management of those asymptomatic women was unclear ***(Vasibuch et al., 2010).***

The efficacy of vaginal progesterone gel was investigated in reducing the rate of preterm birth prior to 33 weeks among women with sonographic evidence of short cervix but were otherwise asymptomatic ***(Hassan et al., 2011).***

Throughout the world, clinicians prescribe progesterone to women who achieve a pregnancy after either IVF or ICSI. However, supplementation with progesterone in early pregnancy has never been subjected to randomized trials documenting any clinical benefits ***(March of Dimes, 2011).***

Aim of the Work

The purpose of this study is to substantiate the recent finding about the effect of vaginal natural progesterone on prevention of pre-term birth after ICSI pregnancies through a randomized controlled trial.

CHAPTER (I)

Pharmacodynamics of Progesterone

Different types of progesterone

Progesterone preparations can be divided into two main groups: natural progesterone, and derivatives, synthetic progesterone and derivatives. Natural progesterone is quickly inactivated when taken orally because of its rapid metabolism in liver and intestine. Therefore, synthetic derivatives were developed to improve bioavailability. There are two main groups of synthetic progestins: (i) the 17 hydroxy progesterone derivatives and (ii) 19 nortestosterone derivatives (Table 1).

Table (1): Different types of progesterone and derivatives

Natural progesterone and derivatives
Progesterone
Dydroprogesterone
Medrogestone
Synthetic progesterone derivatives
17 hydroxyprogesterone derivatives
Hydroxyprogesterone heptanoate
Hydroxyprogesterone caproate
Chlormadinone acetate
Medroxyprogesterone acetate
Cyproterone acetate
19 nor testosterone derivatives
Norethisterone
Norethisterone acetate
Lynestrenol
Ethinodiol diacetate
Norgestrel
Levonorgestrel
Desogestrel
Norgestimate
Gestodene

The latter comprise the 13-methyl gonanes group and 13 ethyl gonanes group. The change in the hormonal spectrum from androgenic to progestational activity is achieved by the removal of the angular methyl group in C19 (19-nor) and the introduction of the ethyl group in C 17a ***(Fatemiet al., 2009).***

Attempts to improve the bioavailability of natural progesterone were investigated because of the limited therapeutic value of synthetic progestin due to side-effects and possible teratogenic effects ***(Kyrouet al., 2012).***

For this reason, long-chain unsaturated fatty acids were used to improve oral progesterone absorption. Reduction of the particle size of progesterone by micronization enhances bioavailability ***(Hargrove et al., 1989; Fatemi et al., 2009).***

However, the micronized form of a single oral dose is inefficient in providing adequate concentrations throughout the day ***(Van der Linden M et al., 2011)*** (Table 1).

Bioavailability

Following the intake of 100, 200 and 300 mg oral micronized progesterone in a fasting condition, the maximum plasma concentration (Cmax) and maximum time for absorption (Tmax) values were found to be 6.5 ± 1.8, 13.4 ± 3.6 and 32.3 ± 7.8 ng/ml after 2.7 ± 1.0, 2.7 ± 2.2 and 2.0 +1.4 h (mean ± SE) respectively (Table II).In addition,

absorption of micronized progesterone was enhanced two-fold in the presence of food ***(Fatemiet al., 2009).***

However, acceptable plasma progesterone concentrations were also obtained following a smaller dose of progesterone (i.e. 100 mg daily) administered via the vaginal mucosae. A maximum concentration of progesterone of 13.97mg/ml can be reached within 3 h in the majority of cases following the administration of100 mg micronized progesterone per vaginally ***(Fatemiet al., 2009).***

Plasma values have been found to be positively correlated with the doses of progesterone delivered by the vaginal route ***(Simon et al., 1993)***, although some researchers did not observe significant differences of plasma progesterone values between the various doses administered by the vaginal route ***(Archer et al., 1995; Gazvani et al., 2012).***

In a comparative study, the area under curve (AUC) values did not differ significantly between a daily dose of 200mg orally and a 400 mg vaginal suppository ***(Norman et al., 1991)*** (Table 2)

Table (2): Some pharmacodynamics characteristic of the various routes of progesterone

Reference	Route of administration	Dose	C_{max} (ng/ml)	T_{max}	Time to return basal value
Nahoul *et al.*, 1987	Oral micronized (Utrogestan®)	200 mg/day	4.70 ± 1.15[b]	3 h	24 h
Devroey *et al.*, 1989	Oral micronized (Utrogestan®)	300 mg/day	7.21 ± 1.57[b]	2 h	6 h
	Vaginal micronized (Utrogestan®)	300 mg/day	8.83 ± 1.88[b]	2 h	8 h
	Vaginal micronized (Utrogestan®)	600 mg/day	13.09 ± 1.49[b]	4 h	8 h
	i.m. progesterone in oil	100 mg/day	71.87 ± 6.73[b]	2 h	10 h
Cicinelli *et al.*, 1991	Intranasal	11.2 mg/day	3.75 ± 0.21[a]	1 h	700 min
Norman *et al.*, 1991	Oral micronized (Utrogestan®)[c]	200 mg/day	28 ± 18.7[a]	3.1 h	24–32 h
	Vaginal suppository (Cyclogest®)	400 mg/day	29.2 ± 53.4[a]	8.1 h	24 h
Nahoul *et al.*, 1993	Oral micronized (Utrogestan®)[d]	100 mg/day	1.5 ± 0.2[b]	2 h	6 h
	Vaginal micronized (Utrogestan®)	100 mg/day	4.7 ± 0.8[b]	6 h	48 h
Simón *et al.*, 1993	Oral micronized (Utrogestan®)[c]	200 mg/day	69.2 ± 31[a]	3.1 ± 2.7	
	Oral micronized (Utrogestan®)[d]	100 mg/day	6.5 ± 1.8[a]	2.7 ± 1.0	
	Oral micronized (Utrogestan®)[d]	200 mg/day	13.4 ± 3.6[a]	2.7 ± 2.2	
	Oral micronized (Utrogestan®)[d]	300 mg/day	32.3 ± 7.8[a]	2.0 ± 1.4	
	Progesterone in oil	50 mg/day	14.3 ± 1.0[a]	8.7 ± 2.0	
Miles *et al.*, 1994	Vaginal micronized capsules	800 mg/day	6.64 ± 1.32[b]	4 h	
	i.m. progesterone in oil	100 mg/day	16.06 ± 1.63[b]	4 h	
Archer *et al.*, 1995	Vaginal suppository.	100 mg/day	14.50 ± 4.61[a]	3 h	
	Vaginal suppository	200 mg/day	14.69 ± 4.26[a]	8 h	
Cicinelli *et al.*, 1991	Vaginal micronized (Geston®)	100 mg/day	5.30 ± 1.04[a]	45 min	24 h
Fanchin *et al.*, 1997	Vaginal gel (Crinone®)	45 mg/every 2 days	3.90 ± 0.40[b]	7 h	37 h
	Vaginal gel (Crinone®)	90 mg/every 2 days	6.32 ± 1.30[b]	7h	43 h
	Vaginal gel (Crinone®)	180 mg/every 2 days	7.47 ± 0.61[b]	7 h	43 h

C_{max} = maximum plasma concentration; T_{max} = maximum time for absorption.
[a]Mean ± SD; [b]mean ± SE; [c]Non-fasting; [d]Fasting

According to a study by ***Van der Linden et al. (2011),*** plasma progesterone concentrations reached 4.7 ± 0.8ng/ml by vaginal administration, but only 1.5±0.2 (mean ± SE) by the oral route following 100 mg of a micronized progesterone intake in fasting condition. The plasma progesterone concentration returned to basal values within 6 h when the oral route was used, whereas progesterone values were still higher than the basal value at the end of 48 h when the vaginal route was used., There are more metabolites of progesterone in blood when using the oral route as a result of extensive metabolism in gut and liver ***(Van der Linden et al., 2011).***

Data show that bioavailability of progesterone is better when the vaginal route is used than when the oral one is used, Progesterone (IM) in oil administration produces higher plasma concentrations which are sustained for longer periods. Bioavailability of oral progesterone is-10% of IM progesterone ***(Van der Linden et al., 2011).***

The bioavailability of progesterone administration by nasal spray has been shown ***(Cicinelli et al., 2000).*** The progesterone administration yielded a mean Cmax of 3.75 ng/ml at Tmax: 1 h by nasal spray ***(Cicinelli et al., 2000)*** (Table II) and a Cmax of 14 ng/ml at Tmax 2 h by rectal administration ***(Chakmakjian and Zachariah, 1987).***

The mean serum progesterone concentrations had been reported as 57.8ng/ml in the patients undergoing cryopreserved embryo transfer given daily 1200 mg sublingual progesterone ***(Fatemi et al., 2009).***

Route of administration

Various routes of administration were developed because of the relatively poor bioavailability and rapid inactivation of progesterone when administered orally. The various routes; I.M., vaginal, intranasal ***(Steege et al., 1986; Cicinelli et al., 2000)***, rectal ***(Chakmakjian and Zachariah, 1987)*** and sublingual ***(Stovall et al., 1996)***, were all investigated. The trans-dermal route is not suitable forprogesterone because of poor dermal absorption and a consequent requirement for high doses ***(De Ziegler et al., 2000).***

The vaginal route presents interesting advantages ***(Smitzet al., 1992; Casanas-Roux et al., 1996; Fanchin et al., 1997; Gazvani et al., 2012).***

Micronized natural progesterone capsules formulated for oral use can be administered vaginally ***(Zarutskie et al., 2009).***

Some of the advantages of the transvaginal route are: (i) avoidance of local pain; (ii) avoidance of first pass hepatic metabolism; (iii) rapid absorption; (iv) lack of undesirable side-effects, e.g. the hypnotic effect; (v) relatively high

bioavailability; (vi) the possible reservoir effect of the vagina for a suppository and (vii) most importantly a-local endometrial effect (known as the uterine first pass effect).

Side-effects

The side-effects are more prominent with regard to the oral synthetic progestins of these, 19 nor pregnane derivatives may have side-effects, e.g. masculinization, decreased high density lipoprotein concentrations ***(Larsson-Cohn et al., 1981),*** luteolysis and possible teratogenic effects ***(Van der Linden et al., 2011).***

As a result of their androgenic properties, in addition to mood changes and depression. The major side-effects of oral micronized progesterone are sedative and hypnotic effects because the metabolites of progesterone effect the affinity of the γ-aminobutyric acid (GABA) receptor in the central nervous system ***(Kyrou et al., 2012).*** Other side-effects of oral micronized progesterone are fatigue, dizziness, headaches, faintness and urinary frequency ***(Zarutskie et al., 2009).***

Local pain has been frequently reported when the I.M. route is used. Cold abscess may occur with the prolonged use of the I.M. route. These side-effects occur seldom as a result of the vaginal route of administration. More specific for the vaginal route are vaginal irritation, vaginal discharge and

dyspareunia. Pervaginally, progesterone can be administered in larger doses, e.g.800mg, without any reported side-effects ***(Miles et al., 1994; Stovall et al., 1996)*** have not reported any side-effects in the patients undergoing cryopreserved embryo transfer following 1200mg sublingual progesterone administration. Progesterone administration by nasal spray has not resulted in any nasal irritation ***(Fatemi et al., 2009).***

CHAPTER (II)
Clinical Use of Progesterone in ART

Ovarian stimulation regimens without GnRH agonist cycles The quality of the luteal phase after ovarian stimulation and the impact of follicle aspiration on the luteal phase have been investigated. Some authors have suggested that follicle aspiration may result in granulosa cell depletion leading to a negative effect on the luteal phase ***(Van der Linden et al., 2011).***

Other studies did not support this hypothesis ***(Zarutskieet al., 2009).*** Whether the luteal phase must be supported in stimulated cycles without using GnRH agonists, remains an open question.

Most studies have failed to demonstrate a clear benefit from luteal support in ART cycles with stimulation regimens, e.g. human menopausal gonadotrophin (HMG) either alone or combined with clomiphene citrate (CC) ***(Poison et al., 1992;Gazvani et al 2012).***

In in-vitro fertilization (IVF) cycles using CC + HMG, there was no difference between the group with supplementation using 50 mg progesterone in oil (8%) and the group without supplementation (14%) in terms of pregnancy rate ***(Belaisch-Allart et al., 1987)*** investigated the effect of luteal supplementation with dydrogesterone on pregnancy rates in CC-HMG cycles. Although the pregnancy

rates improved in the dydrogesterone group, the differences between the supplemented and the non-supplemented group were not statistically significant.

Van Steirteghem et al. (1988) did not demonstrate any improvement on the basis of 50 mg progesterone I.M. in the patients treated by IVF or gamete intra-Fallopian transfer (GIFT) using CC-HMG cycles (26.4% in the supplemented group compared with 19% in the nonsupplemented group).

Some authors have reported that ovulation induction protocols (which did not include GnRH agonist) had to be supported anyway in the luteal phase, because they observed an absolute fall in salivary and plasma progesterone concentrations ***(Van der Linden et al., 2011).***

Plasma progesterone values were found to be higher following luteal HCG supplementation than following 50 mg daily progesterone administered vaginally in non-conception IVF cycles ***(Van der Linden et al., 2011).***

Significantly higher pregnancy rates were found between a group supplemented with a 200 mg vaginal pessary and a non-supplemented group in GIFT cycles treated by HMG + CC ***(Kyrou et al., 2012).***

Triggering of ovulation by GnRH agonist instead of HCG may produce short luteal phase in the absence of hormonal luteal support ***(Lanzone et al., 2006).***

The luteal support by HCG administration overcomes luteal phase inadequacy after GnRH agonist-induced ovulation in gonadotrophin-stimulated cycles ***(Fatemi et al., 2009).*** In the current literature there is insufficient evidence to recommend the routine use of progesterone to support the luteal phase after oocyte retrieval in ART cycles treated by CC-HMG or HMG alone ***(Kyrou et al., 2012).***

GnRH agonist cycles

It was clearly shown that serum luteinizing hormone (LH) concentrations and the pituitary gonadotrophin reserve remain suppressed for at least 10 days after the ovulatory stimulus in GnRH agonist cycles (desensitization protocols).

The premature luteal regression in GnRH agonist cycles may be (at least partly) attributed to prolonged desensitization of the gonadotrophs by the agonist. For this reason, the use of luteal supplementation was advocated for GnRH agonist stimulated cycles ***(Van der Linden et al., 2011).***

Two prospective randomized studies ***(Smith et al., 1989; Belaisch-Allart et al., 1990)*** demonstrated the necessityfor luteal support after GnRH agonist-HMG stimulation forIVF. Further studies to explain the

underlyingphysiopathological mechanisms showed a reduction of thepituitary gonadotropin secretory capacity during the luteal phase in both long and short protocols ***(Smitz et al., 1992))***

Luteal support by HCG significantly improved clinical and ongoing pregnancy rates as compared with oral micronized progesterone 400 mg, especially in short protocols with GnRH agonist and to a lesser degree in long ones ***(vander Linden et al., 2011).***

HCG supplementation was found to be superior to I.M progesterone for the ultrashort protocol using GnRH agonist/HMG.

In the study by ***Golan (1993)*** the conception rate was seven out of 30 using HCG and one out of 26 in I.M. progesterone groups, although the luteal progesterone concentrations were not significantly different.

Other papers, however, reported similar pregnancy rates between groups using either HCG or progesterone given I.M. in IVF using aGnRH agonist ***(Claman et al., 1992; Gazvani et al., 2012).***

Improved implantation and clinical pregnancy rates and lower abortion rates were seen in the 600 mg daily vaginal progesterone group as compared with the50 mg daily I.M. progesterone group despite the lower serum