Oxytocin and vasopressin V1A receptors as new therapeutic targets in assisted reproduction

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Present by R5 郭恬妮
Introduction

- IVF/ET: averaging at about 30% live births per treatment cycle
- Embryo transfer is an independent factor affecting the outcome of the treatment
- The determinants of success of embryo transfer involve the quality of embryo(s) and uterine receptivity, the quality of the intrauterine environment
Uterine contractions constitute one of and the most fundamental components of uterine receptivity → important role in embryo implantation

Excessive uterine contractions → might expel embryos from the uterus

Up to date, treatment strategies to reduce uterine contractions before embryo transfer such as the use of beta agonists or non-steroid anti-inflammatory drugs have not been shown to provide sufficient benefit.
Oxytocin and vasopressin V1A antagonists represent a novel class of drugs developed for patients experiencing threatened premature birth.

decrease intrauterine production of PGF2α → reduction of uterine contractions and improvement of uterine blood supply → potentially beneficial for embryo implantation.

This paper presents a review of what is known about the application of oxytocin/vasopressin V1A antagonists for implantation support in assisted reproduction.
Oxytocin, vasopressin and their receptors

- Oxytocin receptors (OTR) have been found in ovary, testis, blood vessels, heart myocytes, pancreas and kidney as well as on several types of cancer cells.

- Oxytocin: important mediator in the central nervous system, with significant roles in maternal, sexual and social behavior.

- Vasopressin: V1A subtype receptor is found in the uterus, being responsible for contractile responses.

- V1A receptors are also found in vascular smooth muscle cells, platelets and hepatocytes where they mediate contraction, proliferation, hypertrophy of cells and platelet aggregation.
Oxytocin and V1A vasopressin receptors share similar structures as they both belong to the **class I family of G-coupled receptors**.

Binding stimulates phospholipase C activity → releases triphosphoinositol and diacylglycerol, inducing **mobilization of intracellular calcium**.

Calcium triggers phosphorylation of light myosin chains, which in turn promotes contractile activity.

In myometrial cells, activation of OTR → phosphorylation and activation of mitogen-activated protein kinase → increase in cyclooxygenase-2 production → enhances uterine contractions.
Oxytocin evokes the calcium influx through receptor-coupled calcium channels.

Braileanu et al. (2001): vasopressin acting through V1A receptors also increases phospholipase C activity.

In the uterus, oxytocin remains closely associated with another strong uterotonin, PGF2a.

In endometrial stromal and glandular cells, oxytocin enhances the secretion of PGF2a and the expression of PGF2a receptor.
Oxytocin is locally synthesized in the endometrium and in fetal membranes where it stimulates uterine contractions through action on its own receptors and by increasing PGF2a synthesis.

In the animal model, the administration of oxytocin stimulates uterine PGF2a expression → reduction in endometrial blood supply → reduced embryonic survival rate.

Synthesis of oxytocin is strongly influenced by oestradiol.

Oxytocin and vasopressin plasma concentrations increase during the follicular phase, reaching the maximum around the time of ovulation while they decrease during the luteal phase.
- Oxytocin mRNA in the endometrium follows a similar pattern and also reaches its maximum in the mid-cycle phase.

- A relative increase in oestradiol concentrations stimulates the synthesis of oxytocin receptors in the myometrium before labour.

- Kimura et al. (1996): a 300-fold increase in the production of OTR mRNA in pregnant myometrium near term.

- Expression of vasopressin V1A receptors and concentrations of vasopressin do not seem to be affected by steroids and are not altered in pregnancy.
Both oxytocin and vasopressin are involved in induction and maintenance of uterine contractions during labour.

Similarities between oxytocin, vasopressin and their receptors may explain cross reactivity of oxytocin to V1A receptors.

It has been shown that oxytocin may still exert its actions even when OTR are blocked, through action upon the V1A receptors.
Treatment cycles induce an abundant increase in oestradiol concentrations (about 10–20 nmol/l) at the end of ovarian stimulation as compared with less than 2 nmol/l before the ovulation in the natural cycle.

Supraphysiological concentrations of oestradiol induce local (endometrial) production of oxytocin, formation of oxytocin receptors, and – indirectly – formation/release of PGF2α similar to the prelabour status.

It has been shown that uterine contractile activity in IVF cycles is increased by approximately 6-fold when measured before embryo transfer as compared with the situation before ovulation in the natural cycle.
Uterine contractions in treatment cycles

- Uterine contractions play an important role in human reproduction ➔ rapid and directed sperm transport and high fundal embryo implantation.

- In IVF/ET treatments, a progressive decrease in uterine contractions is observed after the egg collection, reaching nearly a quiescent status at the time of blastocyst transfer (5–6 days after egg collection).

- Such a decrease in contractile activity is thought to further augment the higher implantation rates achieved with blastocyst transfers.

- However, the majority of embryos are still transferred on day 2 or 3 after fertilization, during periods of noticeable uterine contractile activity.
The embryo transfer procedure itself is expected to increase the local oxytocin and prostaglandins release.

Any additional manipulation of the vagina or cervix, such as the use of a tenaculum, provides an additional stimulus for oxytocin/prostaglandin release increases in uterine contractions.

Mansour et al.: in more than half of patients having mock embryo transfer with methylene blue dye, the dye was seen to be transported into the vagina after the procedure.

It was also demonstrated that less than 50% of transferred embryos remained in the uterus 1 h after transfer and about 15% of embryos could be found in the vagina after embryo transfer.
Considering the above: uterine contractile activity at the time of embryo transfer and especially fundo-cervical contractions → expel embryos from the uterus.

Fanchin et al. (1998): about 30% of patients undergoing ET have pronounced uterine contractions.

In that group, success rates of IVF/ET treatment were up to 3-fold less compared with the population of patients with ‘silent’ uteri (16% versus 53% of clinical pregnancies).

That could imply that pharmacological inhibition of increased contractions at the time of embryo transfer could be an attractive target for potential treatment.
Oxytocin and vasopressin V1A receptors as a novel target in fertility treatments

- Inhibition of oxytocin and vasopressin V1A receptors → improve uterine receptivity by decreasing uterine contractions, interfering with PGF2a/oxytocin systems and possibly improving endometrial perfusion

- Selective blockade of oxytocin receptors directly halts contractions and decreases PGF2a release in human uterine smooth muscle cells

- Blocking both vasopressin V1A receptors and OTR may be an optimal approach as oxytocin exerts a relatively strong effect on V1A receptors
Currently, only atosiban, which is a combined oxytocin/vasopressin V1A antagonist, is registered for human use.

Only two other antagonists, the peptidyl, long-acting selective oxytocin antagonist barusiban (FE200440) and the non-peptidyl, orally active, mainly V1A vasopressin antagonist relcovaptan (SR49059) have reached the level of clinical trials.

Other drugs or drug candidates of that group are either not progressing in clinical research or at far earlier stages of development.
Atosiban and barusiban

- Atosiban: for threatened premature birth

- Although it is commonly said to be an ‘oxytocin antagonist’, its affinity to vasopressin V1A receptors is higher than to oxytocin receptors (4.7 nmol/l versus 397 nmol/l, respectively)

- Barusiban is a new-generation, peptidyl, OTR-specific antagonist.

- 300-fold more selective for the human oxytocin receptors than the vasopressin V1A receptors

- Its plasma half-life varies between 2.2 and 2.8 h
In preclinical studies: potency, longer duration of action and reversibility as compared with atosiban.

In monkeys, it was effective in maintaining low intrauterine pressure near the end of pregnancy, suppressing oxytocin induced premature contractions and preventing early delivery.

However, in a second-phase clinical trial of women experiencing premature labour, it was not shown to be of satisfactory clinical effectiveness.

In mice, barusiban was confirmed to support embryo implantation (Figure 1).
Figure 1  Influence of oxytocin and the selective oxytocin antagonist barusiban on implantation rate in mice. (A) Implantations were dose-dependently inhibited by oxytocin. (B) Embryo implantations were dose-dependently restored by barusiban.
The original concept of the novel application of atosiban in embryo transfer recipients was first developed in 2004.

before considering the clinical application it was decided to verify its embryotoxic potential.

The preclinical study involved the application of two embryotoxicity techniques: Both failed to detect an embryotoxic effect of atosiban in concentrations up to 50-fold therapeutic blood concentrations.

Tests performed on human spermatozoa also failed to show an adverse influence.
The very first case of clinical application of atosiban prior to embryo transfer was published in 2007 a 42-year-old patient who had previously undergone 8 embryo transfers involving a total number of 12 good-quality embryos.

Atosiban was administered in intravenous infusion lasting 3 h as per license conditions.

As its plasma steady state is reached within the first hour of infusion → embryo transfer was carried out 60 min from the start of the drug administration.

Uterine contractions decreased from 11 contractions per 4 min to 7 contractions per 4 min and decrease in their amplitude (Figure 2).

Therapeutic success (healthy twins delivered 8 months later) encouraged further investigations.
Figure 2  Effect of atosiban on uterine contraction waves in an embryo transfer recipient. (A) Four-minute recording of uterine contractions before the connection of atosiban infusion. (B) Recording of uterine contractions after 1 h of infusion. Dotted circles mark uterine contractions.
To verify its influence on uterine contractions, a multicentre, randomized, placebo-controlled trial has recently been performed on egg donors.

After ovarian stimulation and egg collection, the participants started luteal support (micronized progesterone) and had a mock embryo transfer (introduction of an empty embryo transfer catheter into the uterus, mimicking regular embryo transfer).

The study compared the uterine contractions between patients receiving placebo and those receiving infusions of atosiban or barusiban.

Both antagonists caused reductions in frequency and amplitude of uterine contractions.

Neither barusiban nor atosiban changed the endocrine profile at time of implantation.
Moraloglu et al. (2010) reported a randomized, placebo controlled trial: 37.5 mg i.v. of atosiban infused before and up to 2 h after the embryo transfer in 160 patients → **significant improvement in both implantation rates and clinical pregnancies**.

Implantation rates per embryo transferred were 20.4% versus 12.6% and clinical pregnancy rates per cycle were 46.7% versus 28.9%.

Fewer early miscarriages (16.7% versus 24.4%).

However, the authors did not evaluate uterine contractions.

Large randomized, placebo controlled trial testing the effect of atosiban on uterine contractions and pregnancy and birth rates would provide a more definite answer to the value of this drug for this indication.
Relcovaptan

- Relcovaptan (SR49059) is a non-peptide, orally active vasopressin V1A/oxytocin antagonist
- Administered orally → used in patients with dysmenorrhoea or for prophylactic/maintenance treatment of pre-term labour sufferers.
- Relcovaptan was shown to decrease myometrial contractions in vitro (Akerlund et al., 1999) as well as in vivo (Steinwall et al., 2004a).
- In a clinical study on pre-term labour patients, it significantly decreased uterine contractions as compared with placebo (Steinwall et al., 2005).
- Being predominantly a vasopressin V1A antagonist, relcovaptan has recently been tested in animal models of congestive heart failure
Other oxytocin antagonists

- there are cheaper (although less safe) and equally effective alternatives to oxytocin/vasopressin V1A antagonists such as nifedipine or beta agonists.

- Currently, some initial reports on potential drug candidates list SSR126768A (Serradeil-Le Gal et al., 2004), GSK221149A (Liddle et al., 2008) and (2S,4Z)-N-[(2S)-2-hydroxy-2-phenylethyl]-4-(methoxyimino)-1-[(2-methyl[1,10-biphenyl]-4-yl)carbonyl]-2-pyrrolidinecarboxamide (Cirillo et al., 2003).

- However, their therapeutic applicability still needs to be determined.
Future prospects

- Initial trials indicated that oxytocin and oxytocin/vasopressin V1A antagonists may be clinically useful in supporting implantation following embryo transfer.

- Clinical use of these compounds should be acceptable for IVF/ET patients, even when the drugs need to be administered intravenously.

- Introduction of oral (non-peptide) drugs from this group could enable testing on the longer-term effect of halting the uterine contractions on embryo implantation.
It is however controversial whether such a treatment should be used after the embryo implantation (occurring 1–3 days after the embryo transfer).

The shorter plasma half-life of peptide antagonists such as atosiban as well as the limited duration of treatment should avoid embryonic exposure and therefore might be preferred.

Further trials are needed to establish the clinical value of this class of medications in this novel indication.
- Maximal effect of oxytocin antagonists given at the embryo transfer is observed within the first 3 h after embryo transfer.

- The average cost of atosiban treatment per patient would reach about 150 Euro, which should not vastly affect overall costs of IVF treatment (averaging at 3000–4000 Euro per treatment cycle).

- The safety profile of atosiban has been extensively studied prior to its licensing for pre-term labour.

- Considering the shortness of treatment (3 h versus 2 days in the obstetric indication), the probability of significant side effects of atosiban would be largely reduced.
For registration of any medication supporting embryo transfer, more preclinical (full embryo toxicity data) and clinical (efficacy) studies would be necessary.

Only a randomized, placebo-controlled trial, comparing pregnancy outcome in patients treated with oxytocin or oxytocin/vasopressin V1A antagonists could provide a definitive answer on the value of this application.

With more favourable data appearing on this indication, the academic community and research-oriented fertility centres could become a significant driving force in promoting further trials.
Thanks for your attention!