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Novel regulators of rabbit reproductive functions

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ABSTRACT

This is a review of original data concerning new extra- and intracellular regulators of rabbit ovarian functions. Effects of some hormones including leptin, ghrelin, oxytocin, arginine-vasotocin, endothelin (ET-1), gonadotropin releasing hormone (GnRH), adrenocorticotropic hormone (ACTH), growth factors such as insulin-like growth factor-I (IGF-I), epidermal growth factor (EGF), nuclear peroxisome proliferator-activated receptor gamma (PPARγ), pharmacological regulators of some protein kinases such as protein kinase A (PKA), mitogen-activated protein (MAP) kinase, cell division cycle protein 2 homolog (CDC2 kinase, CDK), tyrosine kinases), and plant molecules (resveratrol, rapamycin) on the functions of ovarian cells (proliferation, apoptosis, secretory activity, expression of some protein kinases) and reproductive end points (blood level of reproductive hormones, ovarian morphology, number of ovulations, embryo yield and quality, number and viability of offspring), and their possible interrelationships and practical application in rabbit breeding are reviewed.

1. Introduction

Progress in reproductive biology, assisted reproduction and animal production (including rabbit production) is due to improvement in understanding and application of the regulators of reproductive processes. The most well known and potent regulators of reproduction are hormones, growth factors and mediators of their action including cyclic adenosine monophosphate (cAMP), and cyclic guanosine monophosphate (cGMP), protein kinases (Sirotkin et al., 2010c; Sirotkin, 2011), and plant molecules, which can have either regulatory and/or protective properties (resveratrol, rapamycin) (Kolesarova et al., 2012; Kadasi et al., 2012a,b). Rabbits have high reproductive potential, therefore they are widely used as a model in basic and applied reproductive biological studies. Furthermore, the profitability of commercial rabbit farms has increased in recent years due primarily to improvements in genetic selection and management of reproduction (Dal Bosco et al., 2011). Progress in rabbit production is associated with the improvement in studies and application of reproduction regulators. Application of hormones is common in reproduction in modern large-scale rabbit farming (Dal Bosco et al., 2011). The most well-known inductors of rabbit ovarian follicle development and ovulation are analogues of follicle-stimulating and luteinizing hormones, equine chorionic gonadotropin (eCG), human chorionic

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gonadotropin (hCG), as well as analogues of GnRH (Sirotkin et al., 2008a,b, 2010b; Dal Bosco et al., 2011). Other hormones and related regulatory molecules, which could mediate, modify and replace natural and artificial regulators (growth factors, protein kinases, *etc.*) are less studied in this respect, and their application is limited by a lack of basic studies. Nevertheless, they could be potent regulators of rabbit ovarian functions, that promote further progress in management of reproduction in rabbits and other species including farm animals and humans.

2. Gonadotropins and GnRH

Gonadotropins (FSH, LH and their analogues, eCG and hCG), GnRH and its analogues are the most known and widely used promoters of rabbit ovarian follicular growth and ovulation (Sirotkin et al., 2008a,b, 2010b; Dal Bosco et al., 2011). Although the mechanisms of action of these substances are not fully elucidated, FSH, LH, and GnRH can exert their effects *via* stimulation of rabbit ovarian cell division, survival, and metabolism by promoting accumulation of cAMP response element-binding protein (CREB-1) transcription factor (Laukova and Sirotkin, 2007), release of steroid hormones (Nitray et al., 1992; Sirotkin et al., 2008c, 2009b; Chrenek et al., 2010), oxytocin, vasopressin, and cGMP (Nitray et al., 1992), and decrease of the IGF-I output (Sirotkin et al., 2008c) (Figs. 1 and 2). All these effects of gonadotropins were more evident in cycling than in non-cycling or pregnant animals (Nitray et al., 1992) suggesting the existence of cycle-dependent changes in the responsiveness of rabbit ovaries to these stimulators.

Gonadotropin releasing hormone has been shown to exert a mixture of effects on the gonads, causing either inhibitory or stimulatory effects on ovarian cellular steroid output depending on the species (Ramakrishnappa et al., 2005). In rabbits, GnRH agonist directly affected the ovary by inducing ovulation in both hypophysectomized animals and in *in vitro* perfused system (Koos and LeMaire, 1985).

Recently, it has been demonstrated the presence of GnRH and its cognate receptor, (GnRHR type I), in corpora lutea (CL) of pseudopregnant rabbits (Zerani et al., 2010) and their post-receptorial mechanisms, as well as the effects of GnRH on the production of progesterone, prostaglandins (PGE2, and PGF2α), and on the enzymatic activities of nitric oxide synthase (NOS) and cyclooxygenases (COX-1 and COX-2), the enzymes involved in the regulation of rabbit CL life span (Boiti et al., 2000, 2005; Zerani et al., 2007). Apparently, GnRHR-I mRNA and protein abundance did not change at different luteal stages, suggesting that in the rabbit this receptor is not dynamically regulated during the CL lifespan (Zerani et al., 2010).

This study confirms that also the ovary of rabbits expresses GnRH in the cytoplasm of different cell types, including the luteal cells, independently of the luteal stage examined. The presence of GnRHR-I and GnRH, similarly to what has been found in other species (Zerani et al., 2012), supports a direct role of GnRH in the regulation of luteal functions in rabbits (Fig. 1). Positive immune reaction for GnRHR-I was also observed in other rabbit ovary cells such as oocytes, follicular and thecal cells, thus indicating the potential paracrine and/or autocrine role of the GnRH/GnRHR system in modulating ovarian function. GnRH has a direct role in the down-regulation of rabbit CL progesterone production. In fact, *in vitro* experiments (Zerani et al., 2010) showed that buserelin, a GnRH agonist, reduced progesterone production by mid and late CL collected at days 9 and 13 of pseudopregnancy, whereas this steroid synthesis was not affected in early CL. Many studies indicate that GnRH, complexing with cognate receptor, mainly activates phospholipase C (PLC) *via* Gq/11 family G proteins; in turn, PLC catalyzes hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol trisphosphate (IP3) and diacylglycerol (DAG) (Millar, 2005). The above cited study confirmed that this GnRH post-receptorial mechanism is present also in the rabbit CL. Indeed the antagonists for IP3 and DAG, and the inhibitors for PLC counteracted the effects of the GnRH agonist buserelin.

3. Nonapeptide and vasoactive hormones

Nonapeptide hormones (oxytocin, vasopressin, vasotocin) and endothelin-1 (ET-1) can be important regulators of reproductive processes (Boiti et al., 2005, 2007; Sirotkin, 2011). In our experiments on rabbits, oxytocin affected peripheral blood steroid hormone levels and increased the viability of the offsprings, but induced the ovarian follicular atresia, reduced ovulation, pregnancy rate, and embryogenesis (Balazi et al., 2012, 2013). Treatment of cultured rabbit ovarian granulosa cells with arginine-vasotocin increased the release of progesterone and estradiol, but not that of oxytocin, vasopressin or the synthesis of cGMP (Nitray et al., 1992).

Genes for ET-1 and for both its receptor subtypes, ETA and ETB, were found to be expressed, although at different levels, in CL of rabbits throughout the course of their life-span, from early- to late-luteal stages across CL demise up to day 22 of pseudopregnancy. In the rabbit, the luteolytic action of ET-1 *in vivo* was clearly dependent on CL age. Four- and six-day-old CL remained unaffected by the same luteolytic dose of ET-1, as that which caused a striking reduction of luteal function at days 9 and 12. By profiling progesterone in blood, ET-1-induced luteolysis, when effective at days 9 and 12, progressed similarly to that triggered by PGF2α (Boiti et al., 1998). Furthermore, ET-1-induced luteolytic action *in vivo* was prevented by antagonizing receptors for ET-1 with Bosentan, an inhibitor of endothelin receptor types A and B, or by blocking COX activity with indomethacin.

In vitro, ET-1 inhibited basal progesterone secretion from CL and stimulated PGF2α release (Fig. 1). All these ET-1-dependent actions were abolished by the ETA-R selective antagonist, BQ123, and not by the ETB-R antagonist. The luteolytic action of ET-1 was also counteracted by treatment with COX inhibitor, consistently with *in vivo* findings.

Further studies support the hypothesis that PGF2α may regulate luteolysis through intraluteal activation of the renin–angiotensin/ET-1 systems in CL of rabbits that have acquired luteolytic competence (Boiti et al., 2007).