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A REVIEW OF LUTEOLYTIC AND LUTEOTROPHIC EFFECTS OF PROSTAGLANDINS ON THE CORPUS LUTEUM OF PSEUDOPREGNANT RABBITS : SOME *IN VIVO* AND *IN VITRO* INSIGHTS *

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ABSTRACT : For many years prostaglandin E2 and prostaglandin $F_{2\alpha}$ have been known to exert luteotrophic and luteolytic actions on the corpus luteum (CL) of most mammalian species. Although the modalities by which the CL regulates normal reproductive cycles and maintenance of pregnancy are basically similar, increasing evidence suggests that several unique mechanisms regulating the growth and regression of CL exist between different species. In this respect, the corpus luteum of rabbits has not been as extensively studied as that of other farm or laboratory animals and very often the experimental data conflict. This reviews presents what is currently known about the actions of these two prostaglandins by critically examining their *in vivo* and *in vitro* effects. In non-pregnant and normally cycling animals, uterine $PGF_{2\alpha}$ causes the CL to spontaneously regress at approximately 14-16 days post-ovulation depending on the species. Exogenous $PGF_{2\alpha}$ administration may be used to cause CL regression and thus control the ovarian cycle. In the rabbit, however, because it does not have a defined cycle, this luteolytic mechanism comes into play only in the case of pseudopregnancy or pregnancy. The $PGF_{2\alpha}$ analogue, alfaprostol, will reduce the diestrous phase at day 9 of pseudopregnancy In pregnant rabbits, $PGF_{2\alpha}$ (and its synthetic analogues) is the hormone of choice for induction and synchronisation of kindling, having beneficial effects on postpartum fertility.

RÉSUMÉ : Revue des effets lutéolytiques et lutéotrophiques des prostaglandines sur les corps jaunes des lapines en pseudogestation : quelques approches *in vivo* et *in vitro*.
Depuis de nombreuses années, les effets lutéotrophiques et lutéolytiques exercés par les prostaglandines E2 et $F_{2\alpha}$ sur les corps jaunes (CL) de la plupart des mammifères sont bien connus. Bien que les modalités qui permettent aux CL de réguler les cycles normal de reproduction et le maintien de la gestation soient similaires, un nombre de plus en plus grand d'évidences suggère que plusieurs mécanismes particuliers, régulant la croissance et la régression des CL, existent chez différentes espèces. En ce qui concerne les lapines, les corps jaunes n'ont pas été aussi largement étudiés que ceux d'autres animaux d'élevage ou de laboratoire, et les résultats expérimentaux sont très souvent contradictoires. Cette revue actualise les connaissances concernant les actions de ces deux prostaglandines grâce à l'examen critique de leurs actions *in vivo* et *in vitro*. Chez les femelles non gravides et ayant un cycle normal, la $PGF_{2\alpha}$ utérine produit une régression spontanée des CL approximativement au 14-16ème jours après l'ovulation selon les espèces. L'administration de $PGF_{2\alpha}$ peut être utilisée pour provoquer la régression des CL et contrôler ainsi le cycle ovarien. Cependant, chez la lapine qui n'a pas de cycle défini, ce mécanisme lutéolytique entre en jeu seulement en cas de pseudogestation ou de gestation. L'alfaprostol, un analogue de $PGF_{2\alpha}$, peut réduire à 9 jours l'anoestrus de pseudogestation. Chez la lapine, $PGF_{2\alpha}$ (et ses analogues synthétiques) est l'hormone de choix pour l'induction et la synchronisation des mise-bas, avec un effet bénéfique sur la fertilité post partum.

INTRODUCTION

Extensive literature exists that describes the role of prostaglandins (PG) in many physiological and physio-pathological systems. Prostaglandins, a family of biologically active lipids found in many body tissues, are also involved in nearly all phases of the endocrine system regulating reproductive functions. In the female, their actions span from gonadotrophin secretions by the pituitary to control of the ovulatory process, from stimulation of uterus and induction of abortion and term labor to embryo implantation (BEHRMAN, 1979). Because of the great number of studies in the field of reproduction, I will limit my attention only to prostaglandin F2α ($PGF_{2\alpha}$) and prostaglandin E2 (PGE2) by focusing on their actions upon the corpus luteum (CL) of the rabbit, using representative examples from the literature and our own data. Obviously, dealing with the actions of $PGF_{2\alpha}$ and PGE2 on the CL, I will refer to their more relevant effects on progesterone production, especially their respective luteolytic and luteotrophic actions.

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The corpus luteum

The CL is a transient endocrine gland, which is extremely important for the regulation of normal reproductive cycles and in maintenance of pregnancy in several mammalian species. Although the modalities by which the CL controls the aforementioned functions are basically similar in most mammalian species, increasing evidence suggests that several unique mechanisms regulating the growth and regression of CL exist between different species. The CL, formed from the Graafian follicle following ovulation, contains both luteal and nonluteal cells. In most species, the luteal cells include small (< 20 μm) and large (20-30 μm) cells, which are both steroidogenic having the enzymatic array necessary to synthesise progesterone from the substrate cholesterol (JEFCOATE *et al.* 1992). There is still some controversy about the origin of the luteal cells, but it is now well established that during its developmental stages, the CL undergo a continuous remodelling process between these two different cell populations (NISWENDER *et al.*, 1994). In the rabbit, the changes in number and size of small and large dispersed luteal cells during pseudopregnancy were studied by HOYER *et al.* (1986) following enzymatic separation. However, DHARMARAJAN *et al.*, (1988) were unable to distinguish these two types of luteal cells by examining tissue sections of intact CL obtained at almost the same stages of pseudopregnancy. Conversely, the nonluteal cells, which comprise endothelial

cells lining capillaries, connective tissue fibrocytes, macrophages and lymphocytes, are not steroidogenic, but they may play a relevant role in the function and regression of the CL (DEL VECCHIO, 1997).

According to the two-cell theory, the small luteal cells derived from the theca interna and the large ones derived from the granulosa cells lining the follicles, have different properties (NISWENDER *et al.*, 1994). The small steroidogenic cells secrete low basal levels of progesterone but respond to LH with an increase in progesterone release, whereas large luteal cells secrete high basal levels of progesterone but are unresponsive to LH stimulation. It is beyond the scope of this paper to detail the regulation of steroidogenesis, but it is now generally accepted that this process is controlled by several mechanisms in the different luteal cell types in response to many factors produced either locally or arriving via the blood stream. Thus, it is likely that messengers of any cell type may influence the synthesis in any other cell type by paracrine and/or autocrine mechanisms. However, it is now evident that considerable differences exist among species as to the specific characteristic in the mechanisms regulating the synthesis of progesterone by corpora lutea. Rabbit CL were found to have LH receptors and LH-responsive adenylate cyclase, and a cAMP-dependent protein kinase enzyme system, even if the effective luteotrophic significance of LH in rabbits is still debated (MCLEAN *et al.*, 1987). In rabbits, 17β-oestradiol has been identified as the principal luteotrophic hormone, as CL are totally dependent upon it (MCLEAN and MILLER, 1985). That the luteal function of rabbit CL is mostly oestrogen dependent can be easily argued by the finding that exogenous oestrogen prolongs the life span of the CL during pseudopregnancy (BILL and KEYES, 1983), rather than shortening the cycle as in other species. Until recently, it was believed that 17β-oestradiol was produced only by the ovarian follicles, as rabbit CL had no aromatase activity. However, ARIOUA *et al.* (1997) have shown that cultured rabbit luteal tissue from hyperstimulated pseudopregnant animals exhibits an intrinsic aromatase activity, producing 17β-oestradiol.

When examining the cyclical regression of the CL in the process known as luteolyisis, care should be taken to distinguish between "functional" and "structural" luteolysis. Functional regression implies a decline of the progesterone secretory capacity by CL, while structural regression implies disruption of the CL and its involution to form the corpus albicans, composed of connective tissue and collagen. Making a distinction between these two types of regression is not often possible, because luteolysis is a continuous, complex process, which involves not only functional changes taking place within a short time after $PGF_{2\alpha}$ induction, but also structural modifications, which are completed only after several hours and lead to cell apoptosis. By convention, "functional" luteolysis is defined as the time when progesterone levels fall to 50% of controls (EINER-JENSEN and MCCRACKEN, 1976), and "complete" luteolysis when plasma progesterone concentrations drop to the very low values normally found during oestrous (KEHL and CARLSON, 1981). In rabbits, this value of progesterone concentration is usually set below 1 ng/ml. Simply stated, luteolytic factors are those substances which promote luteolysis, either functional or structural, thus inhibiting progesterone secretion, while luteotrophic factors are those substances that promote CL growth and stimulate the production of progesterone. Therefore, much emphasis is placed on progesterone in both cases, because it is the main steroid hormone produced by the CL.

Certainly, progesterone is the most studied hormone produced by the corpus luteum, but several reports have shown that the CL produces oxytocin and large amounts of a variety of PG (HANSEL and DOWD, 1986; SCHLEGEL *et al.*, 1988), which are involved in the development, maintenance, and regression of CL in many species.

Prostaglandins

The most common precursor of PG is arachidonic acid which is released from the lipid bilayer of the cell membrane by the action of enzymes such as phospholipase A_2 (PLA), phospholipase C (PLC) or diglyceride lipase. The free arachidonic acid is then metabolised to different families of PG (PGE, PGF and PGD), prostacyclins and tromboxanes by the cyclooxygenase (COX) pathway or to leukotrienes via the lipoxygenase pathway (BENEDETTO *et al.*, 1987). Prostaglandins are not stored in the cell. Thus, their concentrations in the intercellular space depend upon the availability of precursor fatty acid, which is considered the rate-limiting step in PG synthesis. However, hormones and other factors may promote PG synthesis by binding to specific membrane receptors, thus activating receptor-coupled G proteins, which interact with second messengers to activate PLA. Other signals may activate PLC.

As previously mentioned, PG are widely distributed throughout the body, but their activity is selectively targeted to individual cell types within different tissues by specific binding to plasma membrane receptors. At the cellular level, the PG-dependent effects may be regulated by several factors, such as receptor density (number of receptors per unit weight of tissue) and affinity of the binding (dissociation constants). In general, within the target cell, modulation of PG-induced effects may be regulated below the prostaglandin-receptor level along the signal transduction pathway which, depending on the PG family, includes different stimulatory G proteins, second messenger systems (cAMP, InP_3, DAG, Ca^{2+}), and protein kinases A or C (PKA, PKC). However, it should be remembered that receptor expression for PG might vary in response to up-regulation or down-regulation mechanisms. Today, new tools and technologies applied to molecular biology enable us to evaluate precisely, for example, the changing rates of expression for each prostaglandin receptor type, or even different subtypes (COLEMAN, 1996).

Receptors for both $PGF_{2\alpha}$ and PGE2, distributed mostly on large luteal cells, have been identified in CL from several species (BRANNIAN and STOUFFER, 1991; CHEGINI *et al.*, 1991; RICHARDS *et al.* 1994; FENG and ALMOND, 1996). In the ewe, for example, it has been shown that the small cells contain the majority of LH receptors, while the large ones have the majority of the $PGF_{2\alpha}$ receptors (FITZ *et al.*, 1984; BALAPURE *et al.*, 1989). While several studies have identified receptors for both PGE_2 and $PGF_{2\alpha}$ in luteal cells of different species, similar studies, as far we know, have not been undertaken in rabbits.

Prostaglandin F2α

It was first reported by LOEB (1923) that hysterectomy delayed cyclic regression of CL in guinea pigs. This finding,

subsequently confirmed in several other nonprimate mammalian species (McCRACKEN *et al.*, 1999), demonstrated the central role of the uterus in controlling the functional life span of the CL. It took several years to recognise $PGF_{2\alpha}$ as the luteolysin responsible for cyclic regression of the CL (PHARRIS and WYNIGARDEN, 1969). In several farm animals including rabbits, $PGF_{2\alpha}$ has been identified as the primary uterine factor responsible for luteolysis. In fact, hysterectomy, indomethacin, an inhibitor of COX enzymes which blocks the synthesis of PG, and $PGF_{2\alpha}$ antibodies all lengthen CL lifespan (O'GRADY *et al.*, 1972; CALDWELL *et al.*, 1972.). Prostaglandin F2α administration hastens luteolysis in the pseudopregnant rabbit (GUTKNECHT *et al.*, 1972; BRUCE and HILLIER 1974). However, there is a possibility that a metabolite of $PGF_{2\alpha}$ is actually the systemic luteolytic factor. This is supported by the finding that the metabolite 13-14 dihydro- $PGF_{2\alpha}$ was four times more luteolytic than $PGF_{2\alpha}$ when infused into pseudopregnant rabbit (KEHL and CARLSON, 1981).

In most mammalian species, $PGF_{2\alpha}$ produced by the uterus is directly transferred to the ovary bypassing the systemic circulation by means of the local counter-current system. In the rabbit the transfer from the uterine-ovarian vein to the ovarian artery is anatomically impaired (DEL CAMPO and GINTHER, 1972). The importance of this systemic route in the rabbit is also supported by the finding that no unilateral effect of the uterus on CL can be demonstrated. In fact, bilateral regression of CL occurs after hemihysterectomy (HUNTER and CASIDA, 1967). In pseudopregnant rabbits, plasma progesterone levels begin to decline approximately 12-14 days after ovulation in both intact and hysterectomised animals (SCOTT and RENNIE, 1970; HILLIARD *et al.*, 1974; BROWNING *et al.*, 1980). These findings suggest that the initial decline of CL function does not depend upon the presence of an intact uterus. However, on day 17 of pseudopregnancy complete functional luteolysis is observed only in intact does and is associated with an increase in uterine venous $PGF_{2\alpha}$ levels (LYTTON and POYSER, 1982a). Prolonged luteal function has also been observed in rabbits with induced endometritis (BOITI *et al.*, 1999) thus indirectly confirming the importance of the endometrium for properly timed spontaneous luteolysis. The endometrium of rabbits has been shown to synthesise $PGF_{2\alpha}$, and concentrations of endogenous $PGF_{2\alpha}$ were higher on day 17 than on earlier days of pseudopregnancy (LYTTON and POYSER, 1982b). In blood collected frequently over the time of spontaneous luteolysis from large farm-animals, progesterone decrease is constantly linked to PG (or PG metabolites) pulse release by the uterus (STABENFELDT *et al.*, 1981; KINDAHL *et al.*, 1976). However, the pattern of $PGF_{2\alpha}$ concentrations in the uterine venous blood of rabbits failed to reveal any precise relationship between $PGF_{2\alpha}$ release and initial regression of CL from about day 12-14 of pseudopregnancy (LYTTON and POYSER, 1982a).

It has been hypothesised that $PGF_{2\alpha}$ in sheep is secreted by the endometrium in response to the binding of neurohypophysial oxytocin (OT) with endometrial OT receptors, which are up-regulated by 17β–estradiol following progesterone priming (McCRACKEN *et al.*, 1999). The episodic pulsatile pattern of $PGF_{2\alpha}$ uterine secretion appears to be controlled indirectly by the ovarian steroid hormones 17β-estradiol and progesterone through the hypothalamic OT pulse generator. The model for endocrine control of $PGF_{2\alpha}$ synthesis by endometrial cells during luteolysis as proposed in sheep should be considered with caution in rabbits due to the specific role of 17β-estradiol in this species. In the rabbit, in fact, oestrogen is the major luteotrophin controlling progesterone secretion and exogenous oestrogen, rather than shortening the cycle as in other species, prolongs the life span of CL. (KEYES *et al.*, 1979). Despite the presence of OT receptors on both large and small luteal cells, the physiological relevance of OT remains to be elucidated because inconsistent results have often been obtained in both *in vivo* and *in vitro* experiments. Moreover, the concentration of OT in luteal cells of rabbits is rather limited when compared to that found in domestic ruminant species (WHATES, 1984).

Several hypotheses (HANSEL and DOWD, 1986) have been proposed to explain the decreased progesterone synthesis by CL and luteal regression induced by $PGF_{2\alpha}$ Reduced ovarian blood flow due to the vaso-constrictive action of PGF_2 was advocated in the past, but it has been shown that functional luteolysis occurs several hours before any changes in blood flow can be detected. Other hypotheses include down regulation of LH receptors, uncoupling of the LH receptors from adenylate cyclase, activation of PKC, Ca^{2+} influx, and cytotoxic effects (NISWENDER *et al.*, 1994). Despite intensive investigations, the intra- and intercellular mechanisms by which $PGF_{2\alpha}$ exerts its luteolytic action are not yet well understood. Interestingly, $PGF_{2\alpha}$ -induced antisteroidogenic effects appear to be regulated differently in various species. In the cow, for example, treatment with $PGF_{2\alpha}$ stimulates progesterone secretion from isolated luteal cells (BRUNSWIG *et al.*, 1986).

Prostaglandin E2

In contrast to $PGF_{2\alpha}$, PGE2 appears to function in a luteotrophic or luteoprotective fashion. Several reports indicate that PGE2 is involved in the maintenance of the CL during early pregnancy when progesterone secretion from the CL is necessary for the establishment and maintenance of pregnancy. In fact, if viable embryos are present in the uterus, the CL does not regress under the influence of $PGF_{2\alpha}$ and continues to secrete adequate amounts of progesterone. Additionally, intrauterine infusion of PGE2 lengthened the life span of CL in the pig and prevented luteolysis induced by $PGF_{2\alpha}$ (AKINLOSOTU *et al.*, 1988). Thus, PGE2 may be responsible for the increased resistance of the CL to $PGF_{2\alpha}$ that occurs during early pregnancy.

Many of the effects induced by $PGF_{2\alpha}$ can be antagonized or prevented by PGE2 both *in vivo* and *in vitro*. In ewes, simultaneous infusion of PGE2 and $PGF_{2\alpha}$ prevented the decline in progesterone secretion that was observed in control animals treated with $PGF_{2\alpha}$ alone (HENDERSON *et al.*, 1977). The cytotoxic effect induced by $PGF_{2\alpha}$ was completely reversed when luteal ovine cells were co-incubated *in vitro* in the presence of PGE2 (SILVIA *et al.*, 1984). Endometria and zygotes of pregnant rabbits have been shown to release PG (HARPER *et al.*, 1983). In the rabbit, however, the biological actions of PGE2 on CL have not been as extensively investigated as those of $PGF_{2\alpha}$.

Selection of experimental model

When studying the role of PG in reproduction, it is appropriate to separate their possible physiological actions from the pharmacological effects. In fact, although the latter

can be relevant from a practical point of view, they may not reflect the true involvement of PG in normal physiological processes. This is an intriguing and recurrent problem, as most of the studies on the effects of PG on progesterone production by CL, both *in vivo* and *in vitro,* employ pharmacological doses of PG.

Starting with investigations carried out in whole animal *in vivo* experiments, a number of *in vitro* methods have been used. Each of these approaches offers both advantages and disadvantages as methodological solutions that should be carefully weighed by the researcher.

Working with the intact animal is not as simple as it may appear at a first glance. The *in vivo* luteolytic effect of $PGF_{2\alpha}$ injected intramuscularly, for example, is easily assessed by the evaluation of progesterone concentrations in peripheral blood samples. When mimicking spontaneous luteolysis however, $PGF_{2\alpha}$ should be given by intravenous infusion at small dosages and in discrete pulses. Dealing with prostaglandin *de novo* synthesis by the uterus and/or placenta, the *in vivo* experiments are often elusive, especially when using animals of small size such as the rabbit. One of the main technical difficulties in this case arises from the frequent blood sampling necessary to detect the temporal release patterns of PG or those of their main metabolites. Moreover, the peripheral plasma concentrations of these compounds are very low. For special purposes, ovaries can be auto-transplanted under the skin of the neck next to the jugular-carotid loop, which permits long term access to the vascular supply of the ovary in the conscious, unstressed animal (MCCRACKEN *et al.*, 1969).

The *in vitro* studies employ both isolated intact organs, such as the ovary, isolated parts of organs or tissue (e.g. CL, luteal tissue, follicles) or *in vitro* cultured cells (e.g. luteal cells or granulosa cells). In vitro cultures of isolated dispersed, purified and non-purified large and small luteal cells, harvested from CL following enzymatic dissociation with collagenase are often used to study the PG activity. Less frequently, luteolytic and luteotrophic stimuli are applied to isolated ovaries perfused *in vitro* (DHARMARAJAN *et al.* 1989). This technique was also adopted for longer-term (> 10 h) *in vitro* perfusion of ovaries from rabbits, to study physiological processes such as ovulation, induction of ovulation, and the regulation of CL function. Perfusion of intact ovaries *in vitro* has proved to be a suitable model for the study of these ovarian events. This technique has some advantages over cell culture systems due to the preservation of the three-dimensional structure with intact intercellular communications. Some of the advantages of this technique compared with *in vivo* animal models include the possibility of studying intrinsic function under strictly controlled conditions, and avoiding any negative interactions between organs due to distribution, clearance, and metabolism of the substance under test. Although this technique is valuable to study whole organ functions in greater detail, it requires expensive equipment and extensive experience. More simply, several investigators use intact CL collected from the ovaries of sacrificed rabbits and cultured *in vitro* under static incubation conditions.

In general, results obtained by *in vitro* experiments are simpler to interpret since a large number of disturbing factors can be avoided, but several aspects should be carefully weighed. Some problems may arise from the unavoidable damage to the organs or tissues during their removal and manipulation, substrate availability in tissue culture systems, and loss of cell to cell interactions in dispersed cell culture systems. Although several studies indicate that luteal cells maintain their viability and can be successfully cultured *in vitro*, enzymatic dissociation with collagenase and culture of dispersed luteal cells might alter the properties of these cells with respect to the responsiveness to luteotrophic or luteolytic hormones (MCLEAN and MILLER, 1985). Moreover, independent of the cell type, an increasing body of evidence suggests that cell-to cell communications and intercellular crosstalk, via gap junctions, may have important roles in modulating responses to both luteolytic and luteotrophic hormones (DEL VECCHIO, 1997). In conclusion, the fundamental question as to whether the effects observed *in vitro* would also apply in *in vivo* under physiological conditions often remains unanswered.

Effects of PG by *in vivo* studies in the rabbit

It is widely recognized that, with the exception of primates, $PGF_{2\alpha}$ acts directly on the CL to inhibit progesterone production following binding to specific receptors for $PGF_{2\alpha}$. Different species vary in the length of time following ovulation when the CL becomes responsive to the luteolytic action of $PGF_{2\alpha}$. This may be a few days (4-5) as in the cow, sheep and rat to ten days or more as in the pig. Rabbits are completely refractory to $PGF_{2\alpha}$ injection during both the early- and mid-luteal phases until day 12 of pseudopregnancy (MARCINKIEWICZ *et al.*, 1992). In this species, however, the luteolytic effects due to $PGF_{2\alpha}$ administration are variable and depend not only on the age of the CL, but also on the reproductive state (MARCINKIEWICZ *et al.*, 1992), such as pregnancy or pseudopregnancy, the doses employed, the protocol used, and type of prostaglandin (natural or analogue).

We recently investigated the time-dependent responses of CL to $PGF_{2\alpha}$ in rabbits during the early- and mid-luteal phases of pseudopregnancy (between days 3 and 9) using the analogue, alfaprostol (Gabbrostim, VETEM), given i.m. at a dosage of 200 µg (BOITI *et al.* 1998). On days 3 to 5 of pseudopregnancy, no functional luteolysis was observed, but in the following three days, the number of rabbits responsive to alfaprostol almost doubled from 38% to 83%. By day 9, the treatment was always effective and all animals exhibited functional luteolysis. Our data thus show that young CL in the early-luteal stage are totally refractory to the $PGF_{2\alpha}$ analogue, while those in the mid-luteal phase, between days 6 and 8 develop an increasing responsiveness which parallels the increasing age of CL. The reasons for this time-dependent *in vivo* resistance to $PGF_{2\alpha}$ induced luteolysis remain to be investigated, but are probably inherent to the developmental stage of the CL. However, the greater luteolytic potency of the analogue compared with $PGF_{2\alpha}$ could be due to its increased binding to $PGF_{2\alpha}$ receptors (WAKELING and GREEN, 1981) and/or to its reduced metabolic clearance rate. In fact, it is well known that $PGF_{2\alpha}$ is effectively removed from circulation in a single pass through the lungs (PIPER *et al.*, 1970).

Interestingly, as previously cited, the reproductive state of the doe greatly influences the luteolytic response to $PGF_{2\alpha}$. In fact, while $PGF_{2\alpha}$ injection at day 7 of pseudopregnancy does not cause CL regression, it becomes fully luteolytic when given to pregnant rabbits at day 7 of

gestation (MARCINKIEWICZ *et al.*, 1992). The reason for this different effect is still unknown, but is probably related to gonadotrophin signals conveyed to the CL of pregnant rabbits by the presence of viable embryos in the uterus.

Most of the *in vivo* studies on the luteolytic effect of prostaglandins in rabbits were carried out using natural $PGF_{2\alpha}$ starting at day 9 of pseudopregnancy. In these studies, $PGF_{2\alpha}$ was given by a single injection at a large dose (GUTKNECHT *et al.*, 1972; BRUCE and HILLIER, 1974, MARCINKIEWICZ *et al.*, 1992) or by systemic infusion at lower dosages for several hours (KEHL and CARLSON, 1981). Although all these authors employed supra-physiological doses, no luteolytic effect was demonstrated on rabbits treated before day 12 of pseudopregnancy. In only one report, CARLSON and GOLE (1978) gave a single injection of 1 mg of $PGF_{2\alpha}$ on day 9 of pseudopregnancy that resulted in loss of luteal function, but this finding was never confirmed.

In rabbits, the prostaglandin analogue, etiproston (Prostavet, VIRBAC LAB), has been used by REBOLLAR *et al.* (1992) on day 11 of pseudopregnancy, while NAVA *et al.* (1992) used tiaprost (Illiren, HOECHST), on day 11 of pseudopregnancy after negative pregnancy diagnosis. Few studies have evaluated the dose-dependent luteolytic effects of $PGF_{2\alpha}$ analogues on rabbits. According to REBOLLAR *et al.* (1992), 200 μg of etiproston are necessary to achieve complete luteolysis within 24 hours after treatment. NAVA *et al.* (1992) employed 0.03mg of tiaprost, while 200 μg of alfaprostol were always used for both induction of parturition (FACCHIN *et al.*, 1991) and oestrous synchronisation (FACCHIN *et al.*, 1992; FACCHIN *et al.*, 1998). ALVARINO *et al.* (1995) employed both natural (1-2 mg, Inducel-PG, Lab Ovejero, Leòn, Spain) and synthetic $PGF_{2\alpha}$ (200 □g, Prostavet, Lab Virbac, Barcelona, Spain) to improve the fertility rate of does artificially inseminated on either post-partum day 4 or 11. Preliminary results from our laboratory (data not published) suggest that 100 μg of alfaprostol are highly effective in inducing luteolysis in does treated at day 9 of pseudopregancy. Thus, we will probably see a reduction of the doses of $PGF_{2\alpha}$ analogues, currently employed as recently happened for PMSG dosage recommendations.

To the best of our knowledge, we are not aware of experiments using PGE2 *in vivo* for studying the luteotrophic action of this prostaglandin in rabbits.

Effects of PG by *in vitro* studies

The effects of PG on steroidogenesis in *in vitro* experiments can be easily evaluated by monitoring progesterone output during the incubation period in either static or continuous flow culture conditions. A luteolytic effect is observed whenever progesterone production declines in comparison to untreated controls incubated under the same conditions and a luteotrophic effect when progesterone output increases. As already mentioned, progesterone is the steroid of choice in studying the *in vitro* luteolytic or luteotrophic effects. In fact, at least from day 4 of pseudopregnancy, the rabbit CL lacks the aromatase enzyme necessary to synthesize significant amounts of either androgens or estrogens (GOBBETTI *et al.* 1999), although CL of several other animal species produces these steroids.

Studies of $PGF_{2\alpha}$ actions *in vitro* have given contradictory results regarding production of progesterone. MCLEAN *et al.* (1987) found that neither PGE2 nor $PGF_{2\alpha}$ at a concentration of 0.1-3.0 μg/ml, altered progesterone secretion by dispersed luteal cells obtained from rabbits at day 10 of pseudopregnancy. These discrepancies concerning control of luteal function are probably related to the different *in vitro* techniques used to separate various cell types. Mechanically dissociated luteal cells have been shown to behave quite differently from those separated by enzymatic digestion using collagenase (BROADLEY *et al.*, 1994). O'GRADY *et al.* (1972) found a clear inhibition of progesterone production by tissue sections of CL obtained at day 10 of pregnancy and incubated *in vitro* with $PGF_{2\alpha}$. In this case, however, the discrepancy could be ascribed to the use of CL from pregnant rabbits, which, as previously emphasized, behave quite differently from those of pseudopregnant rabbit. DHARMARAJAN *et al.* (1989) observed that $PGF_{2\alpha}$ did not affect progesterone in the *in vitro* perfused rabbit ovary at days 11 or 18 of pseudopregnancy.

A variety of agents have been proposed to explain the mechanisms of action of $PGF_{2\alpha}$ in regulating functional luteolysis. Recently, we focused our attention on the regulatory actions induced *in vitro* by PG by studying their cellular transduction pathways and the potential role of nitric oxide (NO) as a modulator of steroidogenesis in CL of pseudopregnant rabbits (GOBBETTI *et al.*, 1999; BOITI *et al.*, 2000). Particularly, we were interested in the signalling mechanisms modulating NO production induced by luteolytic or luteotrophic factors after receptor binding of $PGF_{2\alpha}$ and PGE2, respectively. NO is a short-lived radical that transmits signals within and between cells (MONCADA *et al.* 1991). *In vivo* NO is generated by the oxidation of L-arginine in a multistep reaction catalysed by NO synthase (NOS) to yield NO and L-citrulline. In our *in vitro* experimental model, we demonstrated that PGE2 and $PGF_{2\alpha}$ modulate luteal NOS activity and progesterone production differently, depending on the age of the CL. In fact, with day 4 CL, PGE2 was found to depress NOS activity and increase basal progesterone production, while $PGF_{2\alpha}$ had no effect. By contrast, with day 9? CL PGE2 were ineffective, but $PGF_{2\alpha}$ caused a 2.5 fold increase in NO activity and a clear decrease in progesterone output (BOITI *et al.*, 2000). The reasons for this time-related and selective resistance to stimulation with different PG are not known and there is still some controversy about the actual cellular mechanisms protecting prostaglandin-induced regression of the CL in the early period of pseudopregnancy. It is widely accepted, today, that the multiple biological actions of PG exerted on CL, both luteotrophic and luteolytic, depend upon the availability of functional receptors on target luteal cells. The lack of responsiveness of day 4 CL to $PGF_{2\alpha}$ may be due to the reduced number of functional receptors for this prostaglandin or, alternatively, to an increase in the number of receptors for other hormones conveying luteotrophic or antiluteolytic signals. In the same way, the loss of sensitivity of day 9 CL to PGE2 could also be ascribed to removal of PGE receptors or to receptor uncoupling from adenylate cyclase.

The *in vitro* response of day 9 CL to $PGF_{2\alpha}$ is quite different than that observed in the *in vivo* system, when rabbits at day 9 of pseudopregnancy are treated with luteolytic doses of natural $PGF_{2\alpha}$. This different response

may well be explained by the application of the luteolysin in close contact with the tissue.

CONCLUSION

Despite intensive investigations, it is evident that the mechanisms by which CL of rabbits grow and regress during pseudopregnancy are not yet fully understood and further basic studies are necessary. When analysing the comparative aspects of the functions of CL it should be borne in mind that major differences exist between different animal species. Moreover, when comparing the *in vivo* and the *in vitro* data, results are not often clear and unequivocal. For this reason particular caution should be taken with direct extrapolation of results from one species to another. At present, little information is available concerning the signalling mechanism induced by luteolytic and luteotrophic factors during CL development in pseudopregnant rabbits or in different physiological stages. There is little doubt that further knowledge of basic mechanisms of the actions of PG may be of great value to the breeder.

In non-pregnant and normally cycling animals, uterine $PGF_{2\alpha}$ causes the CL to spontaneously regress at approximately 14-16 days post-ovulation depending on the species. Exogenous $PGF_{2\alpha}$ administration may be used to cause CL regression and thus control the ovarian cycle. $PGF_{2\alpha}$ and its synthetic analogues are routinely used to regulate the breeding of farm animals for oestrous synchronisation in the procedure of artificial insemination and in the practice of embryo-transfer. In the rabbit, however, because it does not have a defined cycle, and because ovulation is only induced by mating or hormone treatments with GnRH or hCG, this luteolytic mechanism comes into play only in the case of pseudopregnancy or pregnancy. In rabbits, $PGF_{2\alpha}$ (and its synthetic analogues) is the hormone of choice for induction and synchronisation of kindling (PARTRIDGE *et al.*, 1986; UBILLA and RODRIGUEZ, 1989; UBILLA and RODRIGUEZ, 1990). Several authors have described the beneficial effects of $PGF_{2\alpha}$ on postpartum fertility (REBOLLAR *et al.*, 1989; MCNITT *et al.*, 1997), probably by modifying ovarian steroid hormones (REBOLLAR *et al.*, 1997; NEGATU *et al.*, 1998) and gonadotropin levels (UBILLA *et al.*, 1992). Less explored are the luteolytic capabilities of PG during pseudopregnancy (LAMMERS and PETERSEN, 1987). The $PGF_{2\alpha}$ analogue, alfaprostol, will reduce the diestrous phase at day 9 of pseudopregnancy (BOITI *et al.*, 1988), but in this case an early and accurate diagnosis of pregnancy is absolutely necessary to avoid abortion of pregnant. In the 42-day reproductive cycle scheme, with does artificially inseminated every three weeks, the need for reducing the length of pseudopregnancy (normally up to 15-18 days in rabbits) is not so urgent. Therefore, unless the reproductive rhythm employed in rabbit breeding farms is shortened and ultrasound techniques introduced, the usefulness of early $PGF_{2\alpha}$ treatments remains doubtful.

Recently, in a field survey, we found that infections of the genital system are quite widespread and may be the cause of the relatively high incidence of does having high progesterone levels (P+ does) at the time of insemination (BOITI *et al.*, 1999). Thus, although alfaprostol (200 µg), given 72 or 96 hours prior to AI, has no beneficial effects on fertility (FACCHIN *et al.* 1992), $PGF_{2\alpha}$ treatment might be useful in reducing plasma progesterone levels of P+ does. High levels of progesterone, whatever the cause, have been proven to affect negatively reproductive performance (BOITI *et al.*, 1996).

Finally, it should be emphasised that most of the studies on the actions of PG used the sheep as a model. Therefore due to the differences between species frequently stressed throughout this review, the challenge in the forthcoming years will be to improve basic research in rabbits in order to unequivocally define the physiological function of CL with respect to the endocrinological regulatory PG-dependent mechanisms.

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