4 Physiological functions of PgF2α with regard to reproduction and in particular to commencement of post partum recycling in cows

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In dairy cows, the physiologically anestrous following calving lasts 40-60 days.

Absence of manifestations of estrus, however, does not mean ovarian silence. At 9-15 days post partum, endocrinologically functioning follicles in the antrum of the ovary may be noted. Among these, selection takes place, and, by means of this selection, a dominant follicle becomes a Graafian follicle.

This leads to first ovulation 13-26 days post partum (Callahan et al., 1971), unaccompanied by manifestations of estrus, followed by a luteal phase the duration of which varies in practically all cases. Such luteal phases control the ovarian cycles which are, for the main part, brief (i.e. 8-18 days in 80% of cases), although at times they may be long (26-31 days). Furthermore, these luteal phases are characterized by the absence of manifestations of estrus or by brief estrus (a few hours), as well as attenuated behaviour patterns (figure 1).

FIGURE 1 - *Variations in the plasma concentrations of progesterone during the post partum period. The ovulations and first artificial insemination carried out on observation of first manifestations of estrus*
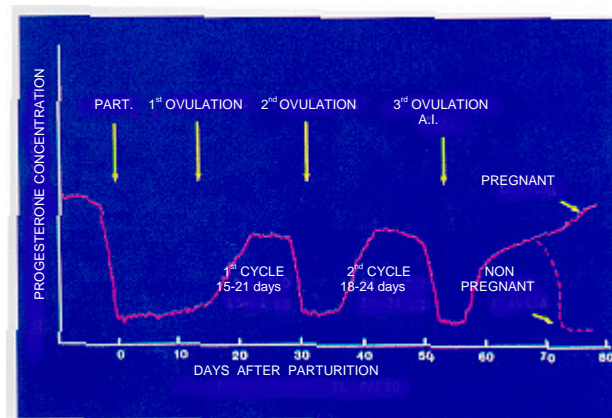
Recovery of ovarian activity after calving does not occur - chronologically speaking - in step with uterine involution or re-epithelialization of uterine caruncles. From the anatomical angle, we may note that the uterus structurally normalizes in 20-30 days post partum. There are also frequent cases of delayed recovery of a physiologically normal size due to endocrine factors (reduction of PgF2α secretion over time, on the part of the uterus itself). Age, too, may be a factor (number of calvings), or environmental conditions (ration and climate).

The period in which recovery of ovarian cycling activity takes place after calving is also most critical. At this stage, diseases may occur not only because of inhibition of follicular development (hypotrophy or ovarian hypoplasia), but also as a result of inhibition of ovulation accompanied by the persistence and abnormal development of the Graafian follicle (ovarian cyst).

The endocrine structures of the follicle (granulosa cells and cells of the theca interna) may persist in intact form and continue to display their activity (mainly production of estrogens). Alternatively, they may undergo luteinization and indice steroid biosynthesis of progesterone.

As noted above, episodic luteolysis may be observed. This may lead to brief or prolonged cycling. Alternatively, luteolysis may be suppressed, leading to persistence of the CL.

In dairy cows, endocrine conditions (which regulate recovery of post partum reproductive activity) and endocrine deficits (which may be indicated as a concomitant factor of pathological states affecting the genital organs during the first 3 months following calving) both depend on:

A *a gradual reactivation of the hypothalamo-pituitary axis which, during the last period of the preceding pregnancy, was inactive;*

B *physiological uterine involution;*

C *"endocrine vitality" of the CL and efficiency of luteolytic processes.*

Before analytically examining the endocrine scenarios characterizing recovery of cycling, it would be fitting at this stage to sum up what is known about the endocrine conditions which determine estrous performance. It should also be borne in mind that, during the post partum period, the constant changes of the first ovarian cycles consist in absence or attenuation of the manifestations of estrus.

ESTRUS

We do not fully understand the determining factors of estrous performance in cows, nor is the knowledge which we do have backed up by experimental data. Nevertheless, experimental data on ewes may be found in the literature. By way of analogy, the data in question may be considered valid for cows.

In ewes, estrus may be induced by combined progesterone and estrogen treatment (Robinson et al., 1956; Moore et al., 1974). Estrogen administration by parenteral route to ewes subjected beforehand to ovariectomy may lead to manifestations of estrus (N.B. dosages of these hormones must be very high).

Pre-treatment of animals with progesterone reduces the load of estrogens necessary for induction of estrus. If repeated pre-treatment of ovariectomized ewes is carried out for long periods of time, the quantity of 17ß estradiol necessary for induction of estrus falls off proportionately to increases in the duration of progesterone treatment.

The minimum plasma levels necessary for induction of estrus are 3 pg/ml (Goodman et al., 1981). This value falls within the physiological range of values noted during diestrus.

The maximum efficacy of treatment with combined progesterone and estrogens is obtained when estradiol 17ß is administered 1-2 days after interruption of treatment with progesterone (Karsch et al., 1980) (figure 2).

FIGURE 2 - *Effect of progesterone (P4) pre-treatment on dosages of Estradiol benzoate (E.B.) necessary for induction of estrus in ovariectomized ewes*
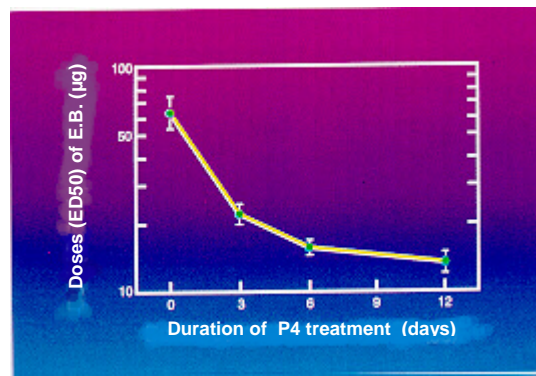
In conditions such as these, the quantity of progesterone administered depends on duration of treatment. The longer the treatment, the smaller the quantity of progesterone need be. The experimental data referred to here show that progesterone acts as a "primer".

The above explains why there are no manifestations of estrus at the time of the first ovulation post partum. At that time, follicular development (excretion of estrogens) is not preceded by a luteal phase.

The short span of the following luteal phase may still determine an absence of estrus, or the attenuation over time of estrous behaviour patterns. Such a condition may involve a number of cycles, according to the duration of the luteal phases of the ovarian cycles.

REACTIVATION OF THE FUNCTIONS OF THE HYPOTHALAMO-PITUITARY AXIS

Recovery of hypophyseal gonadotropin secretory activity after parturition, of course, depends on hypothalamic GnRH secretion and on response efficiency of hypophyseal gonadotropin-sensitive cells. Since it has been known for some time now that ovulation may be induced very shortly after calving by means of administration of hyophyseal gonadotropins (Casida et al., 1943), delayed recovery of cycling cannot be ascribed either to ovarian dysfunction or reduced ovarian gonadotropin receptivity.

It is therefore clear that delayed recovery of the ovarian functions is due to insufficient hypophyseal release of gonadotropins or to a fall in hypothalamic GnRH secretion.

During post partum anestrus, the quantity of GnRH to be found in the hypothalamus is higher than in the cycling cow (Braden et al., 1983). On the other hand, the quantity of LH in the hyophysis is very small indeed (Moss et al., 1985) (figure 3).

FIGURE 3 - *Administration of GnRH by intravenous route induces release of LH in quantities increasing proportionately to length of time since parturition*
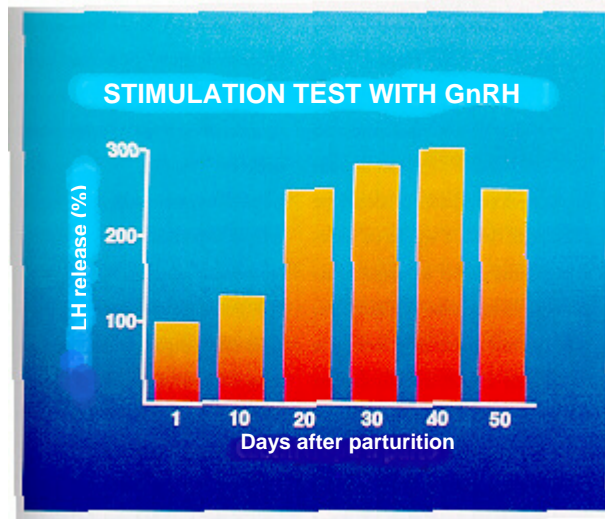
LH deficiency is due to a depletion of this hormone in the hyophysis due to the endocrine scenario of the last period of pregnancy.

Hypophyseal LH synthesis is inhibited by the high progesterone and estrogen levels in the plasma which are a feature of this period of reproductive activity (Tamanini et al., 1986) (figure 4).

**FIGURE 4** - *Endocrine profile during period leading up to parturition*
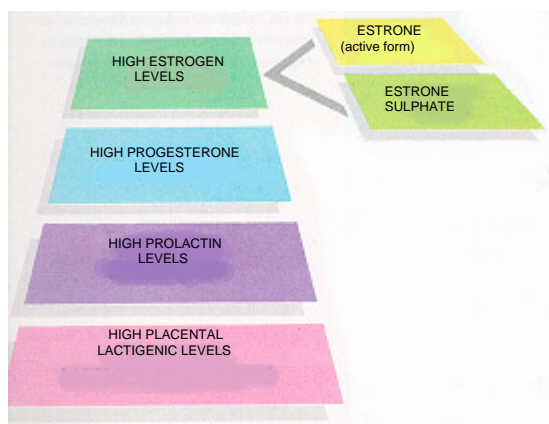
*All of these hormones are **not** of ovarian origin.*
Their combined action inhibits ovarian activity.

Since hypophyseal FSH reserves do not fall during calving, pulsatile GnRH release is followed by pulsatile FSH secretions. These FSH secretions are sufficient to mobilize the follicles of the antrum, to induce follicular growth and to determine full development of follicular LH receptors (Peters et al., 1984). In the meantime, LH is

synthesized and released from the hyophysis in sufficient quantities. Thus, full development of Graafian follicles and subsequent ovulation takes place.

Pre-ovulatory LH peak not only speeds up full follicular development; it also leads to ovulation. See figure 5 for a brief summary of follicular events during the pre-ovulatory LH peak period.

FIGURE 5 - *Endocrine ovulation control flow chart (source: Hafez 1984)*
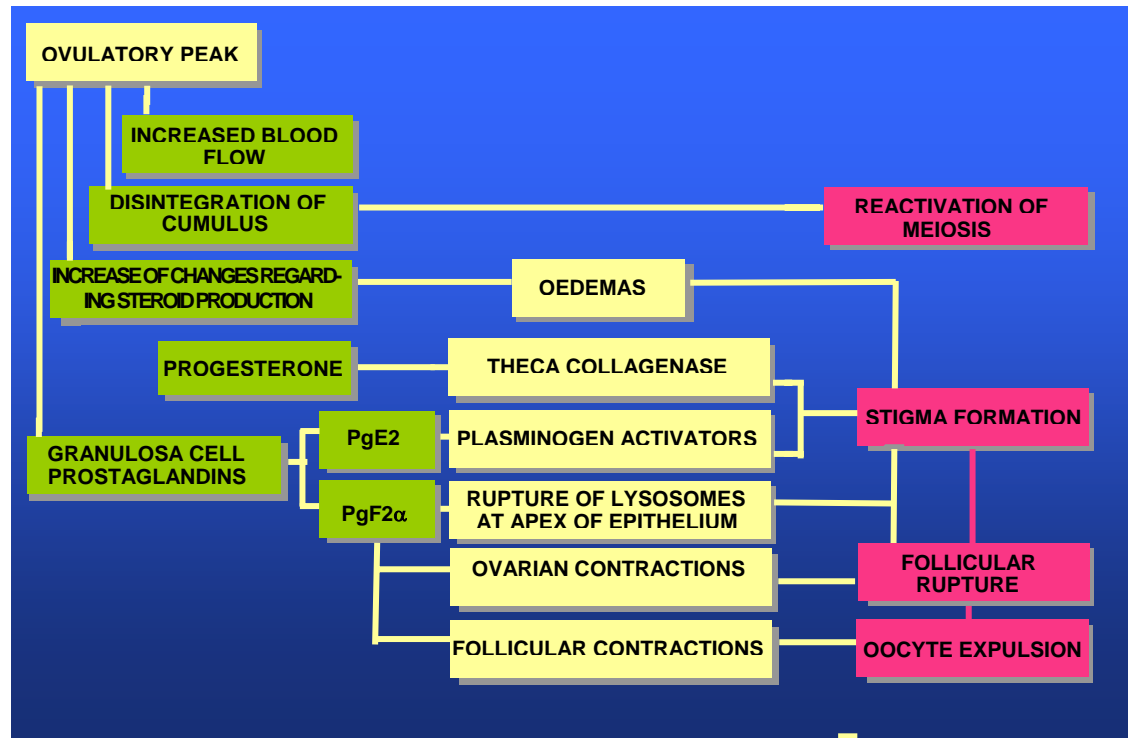
The most important endocrine and structural changes noted in the follicle are as follows:

A *gradual attenuation and inhibition of estrogen synthesis as a result of attenuation of the aromatase activities of enzymes in the granulosa cells;*

B *disintegration of the basement membrane which separates the cells of the theca interna from granulosa cells. This makes direct use of plasma lipoproteins possible for granulosa cell steroid synthesis. These cells are deprived of aromatase activity and therefore steroidal hormone production is limited mainly to progesterone synthesis.*

C *secretion of prostaglandins (PgF2 and PgF2α) by granulosa cells (see figures 6 and 7).*

FIGURE 6 - *Endocrine activity of the theca and granulosa cells of the follicle during follicular growth*
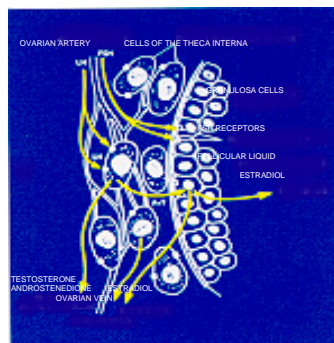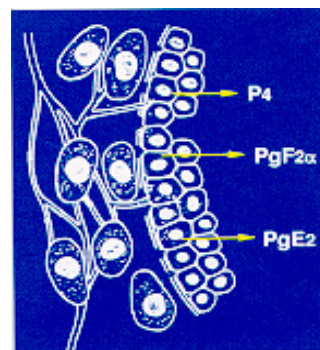
FIGURE 7 - *Structural changes (fragmentation of basement membrane) and endocrine activities of the granulosa cells induced by pre-ovulatory LH peak*

The hormones produced by the granulosa cells during the stage of full follicular development exert a number of local paracrine actions which are essential for the formation of the stigma and for later ovulation. Progesterone

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activates the collagenase enzymes, but the local activity of PgF2α is more complicated and terminates with plasminogen activation. These two enzymes (i.e. collagenase and plasminogen) exert proteolytic action leading to liquefaction of the structures of the theca externa and contribute toward stigma formation.

This activity is enhanced by rupture of the lysosome structures of the epithelium of the apex induced by the local activity of PgF2α. PgF2α continues to activate the myoepithelial structures of the theca externa and ovary (these myoepithelial structures, rhythmically contract, leading to rupture of the follicle) (Hafez, 1984).

Alteration of granulosa cell endocrine activities requires high plasma LH concentrations at the height of the pre-ovulatory peak and for duration of LH peaks.

The time profile for LH concentrations during the preovulatory phase of the cycle is particularly critical: modifications of intensity and duration may inhibit ovulation or lead to regression of the follicle itself (Moore, 1974).

The action exerted by PgF2α during ovulation are not limited to follicular paracrine activities.

The substance (also present in the central nervous system) is one of the hypotalamic and non-hypothalamic chemical synaptic mediators (Thatcher, 1988).

Activation of prostaglandin-dependent synaptic circuits of the hypothalamus leads to increased lypophyseal release of gonadotropins (Furr et al., 1981).

This action furthers follicular development (both during, and on completion) as well as on the later formation of the CL. If PgF2α is administered to cows over the first few days following calving, the first ovulation will be brought forward in time (Villeneuve, 1987).

Furthermore, administration of PgF2α to cows at 25-30 days post partum leads to increased secretion of estrogens on the part of the mature follicle, thereby facilitating ovulation. The above treatment also furthers luteinization of the follicular structures, which takes place after ovulation. An endocrinologically efficient CL is obtained. This CL normally persists for 16-18 days. This means regularization of ovarian cycling and of premature manifestations of estrus (Guibault, 1987).

INVOLUTION OF THE UTERUS

It was noted above that there is often a time lapse between uterine involution and recovery of ovarian cycling.

During the first days prior to parturition, PgF2α plasma concentrations remain high (Kindhal et al., 1984) and there is a direct relationship between these concentrations and the time needed for a reduction of the volume of the uterus (figure 8).

FIGURE 8 - *Reduction of weight of bovine uterus after calving. The weight loss terminates 20-30 days post-calving*
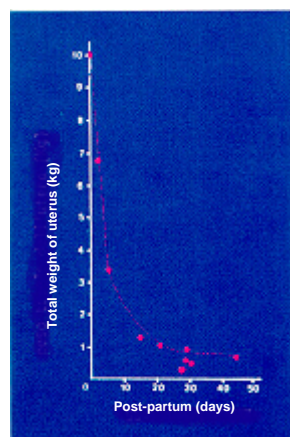
The fact that uterine involution is facilitated by repeated administration of PgF2α during the period following parturition provides indirect evidence of the significance of this compound with regard to the regulation of uterine involution (Lindel et al., 1983).

The PgF2α mechanism behind this action is still the subject of debate. Some researchers note increased uterine motility in cows treated with PgF2α post partum (Ko et al., 1985). However, there are others who cannot confirm this result (Burton et al., 1987).

LUTEOLYSIS

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As was stated above, generally speaking, the length of time of survival of the CL which forms after first ovulation will vary. This is due either to luteolysis commencing too early or to absence of luteolysis.

It is a known fact that luteolysis is induced by release of PgF2α in sufficient quantities on the part of the uterus. Luteolysis is, for its part, controlled by a complex system involving the CL itself.

Luteal cells are developed from the granulosa cells (large cells) and from the theca interna cells (small cells) after ovulation (figure 9).

Large luteal cells secrete not only progesterone but also two polypeptide hormones (oxytocin and relaxin) (figure 10).

FIGURE 9 - *Progress of endocrine activity of granulosa cells when become luteal cells. This progress is affected by insulin*
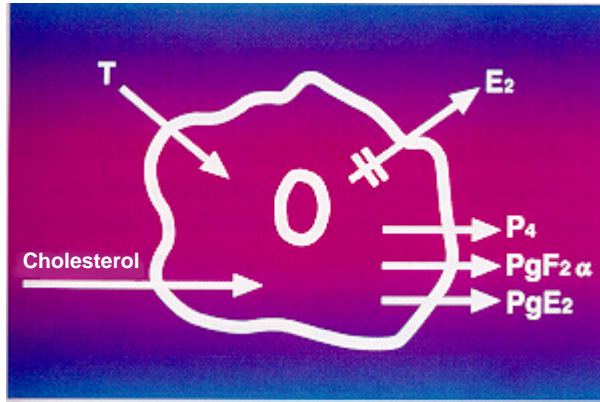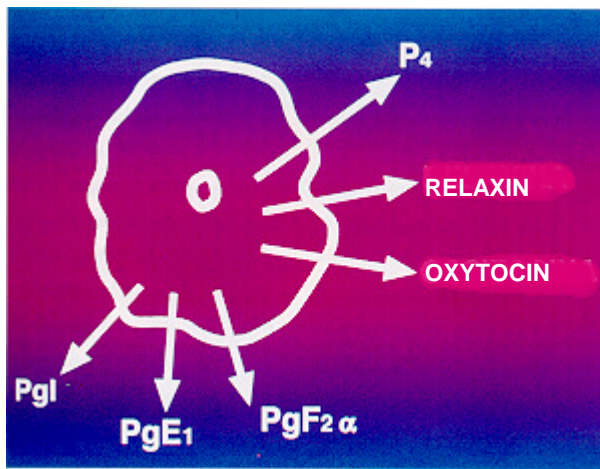

FIGURE 10 - *Endocrine activity of luteal cells derived from granulosa cells (large luteal cells)*

Constant production of progesterone on the part of the CL not only determines structural modification; it activates a process of development of oxytocin receptors in the caruncles. Oxytocin binding with the receptor induces uterine PgF2α secretion (Schallenberger et al., 1984). PgF2α, when released in subluteolytic quantities, in turn, increases CL-released levels of oxytocin (Schams et al., 1985). There is therefore a feedback counter-reaction leading to increased secretion levels of PgF2α until a "critical" level is reached (this level depends on the sensitivity of the vascular structures of the CL) (figure 11).

FIGURE 11 - *Schematic representation of the endocrine mechanisms which induce luteolysis*

P4 = Progesterone
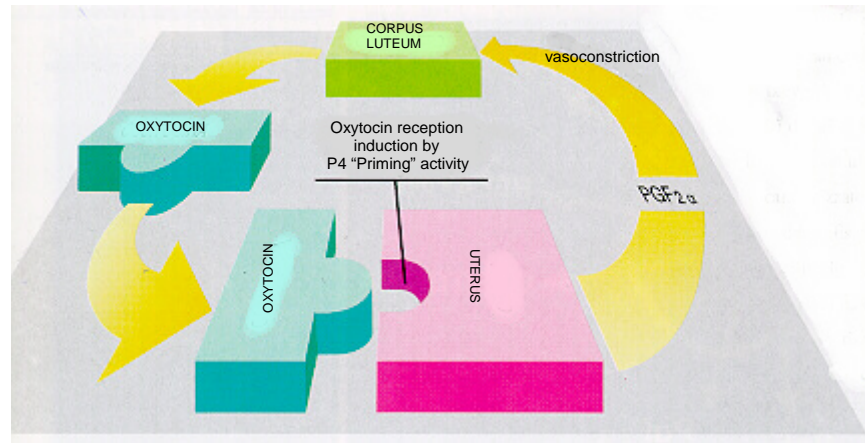
The luteolytic action of PgF2α is in fact a result of intense luteal vasoconstriction, leading to lysis of the luteal vasal system. The CL which forms after the first post partum ovulation is highly sensitive to prostaglandins. It is more than likely that blood concentrations which in normal conditions are subluteolytic will suffice for induction of regression of the CL. This hypothesis is corroborated by experimental data (providing confirmation that luteolysis, in cows with brief estrous cycles, depend on uterine PgF2α secretion) (Troxel et al., 1984; Copelin et al., 1987). It also supports the view that the CL forming after the first post partum ovulations is endowed with greater sensitivity. Nevertheless, it cannot be denied that luteolysis might also be controlled by paracrine mechanisms. Indeed, luteal cells continue to produce PgI, PgE1 and PgF2α. Locally, these prostaglandins directly act on the luteal cells themselves. In a word, PgE activity is, generally speaking, luteotrophic and PgF2α activity luteolytic. The luteal cells of animals displaying brief cycles yield greater quantities of PgF2α than do the luteal cells of those cows displaying normal duration of cycles (Yunfu Hu et al., 1990). There is therefore a local variation in the concentration relation between PgE1 and PgF2α which may facilitate early regression of the CL.

Abnormal persistence of the vitality of the CL during the first cycles following parturition may be ascribed to two factors: pathological conditions affecting the uterus (leading to insufficient PgF2α production on the part of the uterus itself), and endocrine conditions.

Among these latter conditions, a highly significant possibility is that of the occurrence, at the time of milk rising, of constant neurohypophyseal secretion of oxytocin in large quantities.

The high and persistent plasma concentrations of this hormone may lead to uterine down regulation, with regard to oxytocin itself, leading to inhibition of PgF2α release.

Flint et al. (1985) obtained inhibition of CL regression by means of intravenous oxytocin perfusion in ewes during the luteal cyclic phase.

CONCLUSIONS

Luteolysis is not the only physiological action of PgF2α in post partum dairy cows.

During the reproductive phase in question, PgF2α is a factor of uterine involution. The site of action are central and ultimately lead to regularization of hypophyseal gonadotropins. Not only does PgF2α exert paracrine action with regard to pre-ovulatory follicular structures; it also exerts an endocrine activity consisting of control of follicular development (secretion of estrogens), as well as of endocrine efficiency and the vitality (duration) of the CL developing after ovulation.

This last-mentioned activity is related to manifestations of estrus which are particularly evident and prolonged.

The physiological activities of PgF2α during the post partum period provide an explanation for increased reproductive efficiency as a result of treatment of cows with this substance during the period in question.

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