

1 **Review**

2 **Carotenoids in female and male reproduction**

3

4 **Highlights:**

5 - Carotenoids are natural pigments with essential role in promoting tissue growth, female, and

6 male reproductive function

7 - Mammals cannot synthesize carotenoids thereby they need to take them from the diet

8 - Carotenoids have a steroidogenic function and act as regulators of folliculogenesis and

9 oogenesis

10 - Retinol and retinoic acid are essential in the development of mature sperm

11 **Carotenoids in female and male reproduction**

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29 Abstract

30 Carotenoids are among the best-known pigments in nature, confer color to plants and animals,
31 and are mainly derived from photosynthetic bacteria, fungi, algae, plants. Mammals cannot
32 synthesize carotenoids. Carotenoids' source is only alimentary and after their assumption, they
33 are mainly converted in retinal, retinol and retinoic acid, collectively known also as pro-vitamins
34 and vitamin A, which play an essential role in tissue growth and regulate different aspects of the
35 reproductive functions. However, their mechanisms of action and potential therapeutic effects are
36 still unclear. This review aims to clarify the role of carotenoids in the male and female reproductive
37 functions in species of veterinary interest. In female, carotenoids and their derivatives regulate
38 not only folliculogenesis and oogenesis but also steroidogenesis. Moreover, they improve fertility
39 by decreasing the risk of embryonic mortality. In male, retinol and retinoic acids activate molecular
40 pathways related to spermatogenesis. Deficiencies of these vitamins have been correlated with
41 degeneration of testis parenchyma with consequent absence of the mature sperm. Carotenoids
42 have also been considered anti-antioxidants as they ameliorate the effect of free radicals. The
43 mechanisms of action seem to be exerted by activating Kit and Stra8 pathways in both female
44 and male. In conclusion, carotenoids have potentially beneficial effects for ameliorating ovarian
45 and teste function.

46

47 Keywords: carotenoids; retinol; retinoic acid, antioxidants; mammals; veterinary species;
48 folliculogenesis; oogenesis; steroidogenesis, fertility; embryo development; spermatogenesis

- 49
- 50 List of abbreviations
- 51 all trans RA: T-RA
- 52 anti-Müllerian hormone: AMH
- 53 cellular retinoic acid-binding proteins: CRABPs, CRABPI
- 54 cyclic adenosine-3',5'-monophosphate: cAMP
- 55 cytochrome P450 Family 26 Subfamily B Member 1: CYP26B1
- 56 doublesex and mab-3 related transcription factor 1: Dmrt1
- 57 follicle-stimulating hormone: FSH
- 58 glutathione peroxidase: GSH-Px
- 59 gonadotropin-releasing hormone: GnRH
- 60 inducible nitric oxide synthase: iNOS
- 61 inhibitor of DNA binding 4: Id4
- 62 KIT proto-oncogene, receptor tyrosine kinase: KIT
- 63 luteinizing hormone: LH
- 64 mechanistic target of rapamycin: mTOR
- 65 meiotic recombination protein: REC8
- 66 nanos C2HC-type zinc finger 2 and 3: Nanos2, Nanos3
- 67 neurogenin-3: Ngn3
- 68 neurotrophic factor receptor: Gfr α 1
- 69 nitrous Oxide: NO/NOS
- 70 peroxisome proliferator-activated receptor alpha: PPAR α
- 71 phosphoinositide 3-kinase: PI3K
- 72 POU Class 5 Homeobox 1: Pou5f1
- 73 proto-oncogene, receptor tyrosine kinase: KIT
- 74 reactive oxygen species: ROS
- 75 recombination protein: Rec8

- 76 retinoic acid: RA
- 77 retinoic acid-binding proteins: CRABPs
- 78 retinoic acid induced 14: RAI14
- 79 retinoic acid receptors: RAR / RARs / RARalpha / RARbeta / RARgamma / RXRbeta / RXR /
- 80 RARg2
- 81 retinoic acid-stimulated receptor 6 and 8: STRA6, STRA8
- 82 retinol-binding protein: RBP
- 83 spermatogenesis and oogenesis-specific basic helix–loop–helix 1: Sohlh1
- 84 spermatogenesis and oogenesis-specific basic helix–loop–Synaptonemal Complex Protein 3:
- 85 Scp3
- 86 stimulated retinoic acid 8: STRA8
- 87 superoxide dismutase: SOD
- 88 synaptonemal Complex Protein 3: Scp3
- 89 zinc finger and BTB domain containing 16: Zbtb16
- 90

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113 1. Introduction

114 Carotenoids are fat-soluble substances (Maurya et al., 2021) and are among the best-
115 known pigments in nature, confer color to plants and animals (Miyashita and Hosokawa, 2019).
116 About 750 natural pigments are known in this class; they are derived from photosynthetic bacteria,
117 fungi, algae, and plants (Wang, 2014).

118 1.1. Biochemical characteristics

119 The majority of carotenoids are characterized by a polyene structure with eight isoprene
120 units and a carbonaceous skeleton of 40 atoms (Namitha and Negi, 2010) as shown in Figure 1
121 (β -carotene as an example). This skeleton may be characterized by cyclic end-groups and may
122 be complemented with oxygen-containing functional groups.

123 Carotenoids are hydrophobic molecules with very low water solubility. Polar functional
124 groups attached to the polyene chain can change the polarity of carotenoids, which affects their
125 localization in biological membranes and their interactions with various molecules (Jomova and
126 Valko, 2013). Their chemical structure divides carotenoids into hydrocarbons termed carotenes
127 and oxygen derivatives of the hydrocarbons, called xanthophyll (Maoka, 2020). Among the 50
128 kinds of carotenes present in nature (Britton et al., 2004) the best known are α -carotene, β -
129 carotene, ψ -carotene (γ -carotene), and lycopene (Figures 1 and 2); these latter contain only the
130 original hydrocarbon chain with any functional group (Saini et al., 2015).

131 1.2. Source, absorption, and metabolism

132 β -carotene (trans-1,18-bio-[2,6,6-trimethyl-cyclohex1-en-1-yl]-3,7,12,16-
133 tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaen) is considered as a precursor of vitamin A,
134 belonging to the group of provitamins A, like α -carotene and β -cryptoxanthin (Stahl and Sies,
135 2005).

136 Mammals cannot synthesize carotenoids and obtain them from their diet (Walter et al.,
137 2010), for this reason, the main route of carotenoids intake for humans and animals is alimentary.
138 Carotenoids are present in plant foods but various feed additives and dietary supplements
139 containing synthesized carotenoids are also widely used (Hodge and Taylor, 2022).

140 Currently, methods are known for the synthesis of carotenoids from bacteria (*Escherichia*
141 *coli*) (Dong et al., 2017), algae (*Spirulina* or *Arthrospira platensis*) (Gutiérrez-Salmeán et al.,
142 2015), fungi (*Blakeslea trispora*) (Bindea et al., 2018), plants (*Daucus carota*, *Cucurbita* spp.)
143 (Marcelino et al., 2020), as well as with the use of genetic engineering techniques (Walter and
144 Strack, 2011).

145 β -carotene is one of the most important sources of vitamin A, accounting for at least 30%
146 of the dietary vitamin A human intake, for some populations it may represent the only provitamin
147 resource (Weber and Grune, 2012). β -carotene is abundant in fresh and quality forages; however,

148 it is lacking in hay and corn silage (Kamimura et al., 1991). Cattle introduce vitamin A mainly as
149 β -carotene from forages and as supplemented retinol ester in formula feed.

150 Appreciable amounts of these molecules are destroyed in the rumen (Weiss, 1998),
151 therefore the prevalent amount of β -carotene and retinol taken from the diet in this species comes
152 from the intestine (Ikeda et al., 2005).

153 Intestinal absorption and bioavailability of carotenoids is influenced by different types of
154 matrix such as lipids and fibers (Borel, 2003; Mamatha and Baskaran, 2011; Pasquier et al., 1996;
155 Tyssandier et al., 2001). The active ingredients contained in spices also influence the absorption
156 and bioavailability of carotenoids. Recent findings showed the influence of spices active principles
157 on intestinal uptake, bioconversion of retinol, and basolateral secretion of carotenoids at
158 enterocyte level using Caco-2 cells (Shilpa et al., 2021). Eicosapentanoic acid inhibits intestinal
159 β -carotene absorption by down-regulation of scavenger receptor class B, type I expression via
160 peroxisome proliferator-activated receptor alpha (PPAR α) dependent mechanism (Mashurabad
161 et al., 2016).

162 Yuan et al. (2020) reported that more than 40% migrates through the circulatory system
163 into parenchymal organs, particularly liver and ovaries.

164 The molecular structure of β -carotene determines its biological role: it protects cell
165 structures from the transformation caused by aggressive factors such as toxins and oxidants
166 (Gutiérrez-Salmeán et al., 2015) and from the altering effects of reactive oxygen species,
167 contributing to membranes' integrity and functional stability (Aragona et al., 2021).

168 β -carotene in mammals is transformed into retinal and other forms, such as retinol and
169 retinoic acid (RA) (Figure 3).

170 The retinol is esterified and transported to the liver where it is stored (Chew et al., 1984).
171 The biosynthetic steps leading to the biological transformation of β -carotene consist in the retinol
172 being oxidized to retinaldehyde (constitutes the visual pigment rhodopsin) and subsequently in
173 the synthesis of RA (Gottesman et al., 2001). RA is considered the active form of retinol (Jiang et
174 al., 2018).

175 Retinal is necessary for the functioning of the organs of vision while retinol and RA provide
176 tissue growth and regulate the reproductive function (Sergeev et al., 2017).

177 As a result of a complex biochemical synthesis, retinol is transported to the liver in the
178 form of chylomicrons, binding with transthyretin and retinol-binding protein (RBP) and constituting
179 a three elements complex. This complex is the main source of vitamin A that tissues need for
180 their functions (Gottesman et al., 2001). The main distinguishing feature of β -carotene is its ability
181 to accumulate into tissue depots. Further, under the influence of enzymes in the liver and
182 intestines, it turns into vitamin A. This occurs only in the quantities necessary for the body at each
183 physiological stage. It is important to note that β -carotene does not have the toxic effect
184 characteristic of excess or overdose of vitamin A (Klyuchnikov, 2007).

185 RA is a hormone-like compound. It regulates gene expression by activating specific
186 nuclear receptors (RARs), which are ligand-controlled transcription factors. RA and its isoforms
187 are believed to interact with two separate subgroups of nuclear receptors, retinoic acid receptors
188 (RARalpha, RARbeta, RARgamma) and retinoid X receptors (RXRalpha, RXRbeta, RXRgamma).
189 They act as heterodimers with the retinoid X receptor (RXR), constituting RAR-RXR heterodimers.
190 The formation of ligand-receptor complexes will either activate or repress specific target genes
191 by binding to specific response elements present in the proximity of the promoter region (Mohan
192 et al., 2002).

193 There are also non-classical receptors that mediate RA function, namely the peroxisome
194 proliferator-activated receptor beta/delta (Jiang et al., 2018).

195 In particular, RA regulates the expression of genes for several growth factor receptors,
196 including retinoic acid-stimulated receptor 6 (Stra6). Stra6 is a high-affinity membrane receptor
197 for RBP and mediates the transport of vitamin A from the blood into cells (Eroglu and Harrison,
198 2013).

199 1.3. Effect on the reproductive system

200 Recently, several reviews described the biotransformation of carotenoids in animals
201 (Abdelnour et al., 2019; Meza-Herrera et al., 2013) and humans (Jamro et al., 2019; Li et al.,
202 2019; Palini et al., 2014). Hemken and Bremel (1981) highlighting the possible difference in the
203 carotenoid's metabolism between ruminants and monogastric animals. Another review
204 (Damdimopoulou et al., 2019) summarized the current knowledge about retinoids in
205 folliculogenesis and steroidogenesis in post-pubertal mammalian ovaries. D'Ambrosio et al.
206 (2011) thoroughly described the interaction of retinoids with enzymes and carrier proteins, that
207 determines the metabolism of retinoids. Bhardwaj et al. (2021) described the role of natural
208 antioxidant compounds in infertility problems, with a chapter dedicated to carotenoids and vitamin
209 A, as well as how to use them safely. The role of retinoids in the endometrium are described by
210 Jiang et al. (2018).

211 Contrasting results exist on the β -carotene role on reproduction. Some authors (Akordor et
212 al., 1986; Hye et al., 2020; Oliveira et al., 2015) reported the absence of effect on reproduction.
213 Others, described an increase of 13-cis retinoic acid, a teratogenic metabolite for the fetus,
214 following the administration in the mother of a 13-trans retinoic acid excess, a β -carotene
215 derivative (Goldberg, 2011). These results suggested that the administration of these carotenoids,
216 as a dietary supplement, should be monitored, especially in the first trimester of pregnancy
217 (Goldberg, 2011). In contrast, many studies reported that β -carotenes improve reproduction rates
218 (De Bie et al., 2016). Other studies demonstrated that the additional use of β -carotene decrease
219 the number of abortions in sows (Spiegler et al., 2012) and cows (Ascarelli et al., 1985). In
220 addition, there is evidence that the dietary administration of β -carotene was able to reduce
221 embryonic mortality in the presence of vitamin A in gilts (Schweigert et al., 2002). β -carotene

222 improved fertility (Chen et al., 2021), stimulated estrus behavior (Meza-Herrera et al., 2013) and
223 decreased the service period (Gossen and Hoedemaker, 2005), preserving the reserves of luteal
224 retinol necessary to carry out the luteal steroidogenic activity (Schweigert, 2003).

225 In the following sections we will summarize the information about the effect of carotenoids, on
226 mammals' reproductive function. In particular, the role of carotenoids in male and female
227 reproductive processes in species of veterinary interest will be discussed. In particular, the role
228 of the most relevant carotenoids will be discussed with respect to folliculogenesis, follicular and
229 luteal steroidogenesis, oocyte maturation, corpus luteum, embryo and pregnancy for the female.
230 Whereas, for the male, the impact of carotenoids on spermatogenesis and their antioxidant role
231 on sperm quality and parameters will be discussed.

232 2. Females

233 2.1. Folliculogenesis

234 Folliculogenesis is the development of follicles in the ovaries of the female, from primordial
235 to preovulatory. The early stages of folliculogenesis involve molecular mechanisms that target
236 molecules such as the mechanistic target of rapamycin (mTOR), phosphoinositide 3-kinase
237 (PI3K), and those of the mammalian Hippo signaling pathway (Gershon and Dekel, 2020; Shah
238 et al., 2018). The initial stages of folliculogenesis do not depend on gonadotropins. After the initial
239 activation, the synthesis of growth factors, activins and anti-Müllerian hormone (AMH) begins in
240 the follicles and can already act on them both locally and through the hypothalamic-pituitary
241 system. These components are secreted by the ovarian granulosa cells. As the antral cavity
242 forms, follicular growth becomes increasingly dependent on gonadotropins.

243 Various studies proved that RA is involved in the processes of neuro-humoral regulation
244 of the human reproductive cycle (Kawai et al., 2016) and that β -carotene supplementation
245 reduced ovulation failure in bovine repeat breeders (Khemarach et al., 2021). Ikeda et al. (2005)
246 reported that both β -carotene and retinol are present in bovine follicular fluid, with a ratio directly
247 correlated to blood concentration.

248 In contrast to vitamin A, β -carotene concentrations in plasma, corpus luteum and follicular
249 fluid were significantly correlated with each other (Haliloglu et al., 2002) (Table 1).

250 Furthermore, these two forms of carotenoids would be trapped in the follicle by their carrier
251 proteins, thereby explaining the different concentrations found in the follicle (Brown et al., 2003;
252 Schweigert and Zucker, 1988). Moreover, retinol concentrations were higher in larger follicles
253 than in small ones, with an intense immunoreaction in pre-antral follicles (Brown et al., 2003). In
254 contrast, the intrafollicular β -carotene concentration was negatively correlated with the follicle
255 diameter (Haliloglu et al., 2002).

256 In a study of Hidalgo et al. (2005), cows, receiving vitamin A injections and showing normal
257 vitamin A blood concentrations, developed follicles containing high follicular fluid. This study

258 suggested that the follicle is able to incorporate vitamin A, 4 days after its administration. The
259 same authors stated that the volume of fluid collected is not influenced by retinoid treatment
260 (Hidalgo et al., 2005), as reported in pigs (Whaley et al., 2000). In addition, β -carotene
261 accumulated in the corpus luteum (Haliloglu et al., 2002) can be considered as a retinol source
262 in the follicles (Bondi and Sclan, 1984).

263 Kawashima et al. (2012) stated that β -carotene is one of the important nutritional factors for the
264 resumption of reproductive function after parturition in dairy cows.

265 In dairy cows, β -carotene has an immunomodulatory function and decreases the incidence
266 of mastitis (Chew et al., 1982) and placental retention (Michal et al., 1994).

267 Moreover, the conversion rate of β -carotene to vitamin A in granulosa cells is enhanced
268 by follicular growth, and intrafollicular concentration of vitamin A correlated positively with
269 estradiol concentration and follicle diameter (Schweigert and Zucker, 1988).

270 Lower energy supply and plasma levels of β -carotene in the peripartum influence the
271 resumption of ovarian follicular activity after delivery in dairy cows, leading to the ovulatory activity
272 block (Kawashima et al., 2012).

273 In dairy cows, an increased administration of β -carotene in the close-up dry state led to
274 follicles ovulation in the first follicular wave (Kawashima et al., 2012).

275 In contrast, despite β -carotene supplementation (β -carotene 1g/d) and its increased
276 plasma concentrations in the pre-partum period, other authors found no effects on the resumption
277 of ovulatory activity in dairy cows (Kaewlamun et al., 2011).

278 Fujihara et al. (2018) found that RA activated the growth of primordial follicles in cats
279 during co-incubation with ovarian cell culture. However, it did not affect ovarian viability. RA
280 regulates the development of the ovarian follicle, stimulates the proliferation of granulosa cells
281 (Demczuk et al., 2016).

282 The β -carotene addition to the diet of goats outside the reproductive season (50
283 mg/goat/day, from April to May) promoted the active development of follicles (Lopez-Flores et al.,
284 2020).

285 Overall, the general consensus is that the role of β -carotene and its derivatives appears
286 to be positive with respect to follicular growth and maturation.

287 2.2. Steroidogenesis

288 Pituitary gonadotropic hormones interact with follicular components to stimulate follicle
289 development and oogenesis. These hormones act both in an autocrine and paracrine manner
290 (i.e., IGF-1 and 2) (Lopez-Flores et al., 2020).

291 RA (10^{-10} M) and retinol (10^{-8} M) synergistically enhanced the function of follicle-stimulating
292 hormone (FSH) in inducing luteinizing hormone (LH) receptors (Bagavandoss and Midgley,
293 1988). In addition, the combination of these molecules stimulated the formation of cyclic
294 adenosine-3',5'-monophosphate (cAMP) and progesterone (Bagavandoss and Midgley, 1988).

295 However, at higher concentrations, both retinoids suppressed these effects of FSH
296 (Bagavandoss and Midgley, 1988). The endometrium secretes the retinol binding protein (RBP)
297 under the action of progesterone (Trout et al., 1992). Moreover, in human ovarian surface
298 epithelium-C2 cells, RA greatly increased 3 β -hydroxysteroid dehydrogenase mRNA levels
299 (Papacleovoulou et al., 2008).

300 Vitamin A deficiency negatively affects steroidogenesis. The use of retinoids increases the
301 synthesis of progesterone *in vitro* by luteal cells in cattle. A study on cattle luteal cells described
302 that retinoids protect the cytochrome P450, the enzyme involved in the transformation of
303 cholesterol, from free oxygen radicals damage (Brown et al., 2003).

304 β -carotene improved bovine luteal cells steroidogenesis when present at low doses (0.1
305 micromol/l), whereas it was inhibitory at higher concentrations (1 or 2 micromol/l) (Arikan and
306 Rodway, 2000). Moreover, in the same experiment, the encapsulation of β -carotene in
307 cyclodextrin was an efficacious method to provide this molecule to the cells (Arikan and Rodway,
308 2000).

309 Cumulus cells contained endogenously active retinoid receptors and participated to the
310 RA synthesis using the precursor retinol. According to Mohan et al. (2003), retinoids previously
311 administered *in vivo* or *in vitro* can have a receptor-mediated effect on cumulus-granulosa cells.

312 Carotene can inhibit the activation of the estrogen receptors, so confirming its role in the
313 neurohumoral regulation of the reproductive cycle. In cattle orally supplemented with carotene, it
314 was found that this molecule increased the expression of genes involved both in the activity of
315 cellular gonadotropes and in the regulation of gonadotropin-releasing hormone (GnRH) (Haliloglu
316 et al., 2002).

317 Supplementation with different β -carotene doses (0.4, 2, or 10 mg) in cats, during the 8
318 weeks prior to estrus, increased plasma progesterone concentrations between day 6 and 10 after
319 ovulation increasing until day 14 in cats fed a diet with the maximum β -carotene dose (10 mg)
320 (Chew et al., 2001).

321 In mares, synthetic β -carotene supplementation (1 g/d) for 15 days, starting from
322 parturition, enhanced steroidogenesis, leading to a better resumption of cyclicity (Trombetta et
323 al., 2010). In contrast, Watson et al. (1996) showed that ponies did not absorb synthetic β -
324 carotene, with a consequent deficit of ovarian response.

325 The data summarized above indicate the β -carotene and its derivatives exert a positive
326 but dose-dependent role with respect to ovarian steroidogenesis (Figure 4).

327 2.3. Oocyte maturation

328 Various studies reported that vitamin A improved developmental competence of oocytes
329 in cow (Shaw et al., 1995), gilt (Whaley et al., 2000), ewe (Eberhardt et al., 1999) and rabbit
330 (Besenfelder et al., 1993, 1996), even if the latter is a species with physiologically high blood
331 levels of this carotenoid.

332 The oocyte is rich in vitamin A, through its cellular derivative RA (Mohan et al., 2001).
333 Indeed, transcripts of retinoid binding proteins and other RA receptors have been described in
334 bovine oocytes and embryos from the early stages of their development (Mohan et al., 2002,
335 2003). The presence of retinoid nuclear receptor mRNA indicates the existence of a retinoid
336 signaling mechanism in the oocyte (Figure 5). The RA receptors alpha (RAR α), beta (RAR β), and
337 g2 (RARg2) were immune-evidenced in bovine blastocysts (Mohan et al., 2001, 2002),
338 demonstrating that transcripts are translated into proteins.

339 An active RA signaling pathway is fundamental for the onset of oogenesis (Spiller et al.,
340 2012; Teletin et al., 2017). RA is an essential chemical involved in germ cell division, which can
341 initiate meiosis in two ways: the first is RA stimulation of the retinoic acid 8 (Stra8) factor
342 transcription (Damdimopoulou et al., 2019) (Figure 5). Another possible route of meiosis initiation
343 is the activation by RA of the meiotic recombination protein (Rec8) transcription, necessary for
344 the replication of meiotic DNA and, thus, to the successful course of meiotic prophase,
345 (Damdimopoulou et al., 2019).

346 A study (Nasiri et al., 2011) reported that RA increased the rate of oocytes maturation in
347 mice. A 2 to 4 μ M of physiological RA form (all trans RA) improved *in vitro* maturation and
348 development rates of mouse immature oocytes. However, despite these positive effect, the use
349 of higher doses (6-8 μ M) significantly reduced the rate of development and the quality of oocytes
350 (Tahaei et al., 2011).

351 Saadeldin et al. (2019) studied the effect of trans-RA in dromedary cumulus-oocyte
352 complex on *in vitro* maturation. The dose of 20 μ M trans-RA significantly reduced the proportion
353 of degenerated oocytes. There was a significant improvement in the process of oocyte meiosis
354 and extrusion of the first polar body in comparison with both control and experimental groups.

355 Vitamin A plays a unique role in the maturation of the oocyte cytoplasm, in fact, β -carotene
356 can enhance cytoplasmic maturation due to its antioxidant properties (Ikeda et al., 2005). RA also
357 promoted the maturation of the bovine oocytes cytoplasm due to its modulating effect on genes
358 expression for gonadotropin receptors, midkine, cyclooxygenase-2, and nitric oxide synthase in
359 cumulus-granulosa cells (Ikeda et al., 2005). RA via the inhibition of RAR α and inducible nitric
360 oxide synthase (iNOS) expression, activated the nitrous oxide system (NO/NOS) in cumulus-
361 granulosa cells affecting the cytoplasmic maturation of bovine oocytes (Sirsjö et al., 2000) (Figure
362 5). Moreover, RA massively inhibited the expression of iNOS mRNA and NO production in porcine
363 immature oocytes (Hattori et al., 2002).

364 Duque et al. (2002) evaluated the effect of 5 nmol/L RA on *in vitro* pre-maturation and
365 maturation of bovine oocyte-cumulus complexes. Pre-maturation in the presence of RA improved
366 the cytoplasmic competence of *in vitro* matured bovine oocytes.

367 After *in vitro* fertilization and culture, the rate of bovine blastocyst development and
368 hatching was increased in samples matured in the presence of 9-cis-RA (Deb et al., 2011). RA
369 increased the developmental capacity of the oocyte and positively influenced the development

370 and differentiation of the trophectoderm and the maturation of embryos *in vitro* (Hidalgo et al.,
371 2003).

372 In conclusion, the carotenoids exert a positive effect on oocyte maturation, but this effect
373 appears to be dose dependent.

374 2.4. Corpus luteum

375 The yellowish color of the corpus luteum has always been associated with the high
376 presence of β -carotene (Bruggemann and Niesar, 1957; Kirsche et al., 1987) and represents a
377 target of this molecule and its derivatives (Schweigert and Zucker, 1988).

378 In a study conducted in bovine corpora lutea at different stages of the ovarian cycle the
379 authors demonstrated that of β -carotene concentration, but not of retinol, increased with luteal
380 development (Schweigert, 2003). This would be explained by the high metabolic activity of this
381 endocrine gland, in particular, of lipoproteins (lipoprotein bound-cholesterol) involved in
382 steroidogenesis which retain β -carotene (Crociani et al., 2017).

383 Moreover, in cattle, the low concentrations of retinol observed during active
384 steroidogenesis of the corpus luteum suggested a significant role for this molecule in the
385 steroidogenic process. Schweigert et al., (2003) asserted that the dietary β -carotene
386 supplementation in cows helps the corpus luteum to have sufficient retinol reserves to perform
387 steroidogenic activity.

388 High β -carotene concentration is found in the luteal tissue and follicular fluid in cattle
389 (Schweigert, 2003), therefore, β -carotene deficiency negatively affected the sexual cycle: the
390 follicular phase (nympomania) increased and the luteolysis processes was disrupted (Yuan et
391 al., 2020).

392 The intracellular luteal RA concentrations are mostly controlled by cellular retinoic acid-
393 binding proteins (CRABPs). Within the corpus luteum, RBP and CRABPs were observed in large
394 luteal cells, but only RBP was observed in small luteal cells (Brown et al., 2003). In the pig, the
395 CRABPs presence was cycle stage-dependent, the luteal cells in dioestrus expressed CRABP I
396 (Schweigert and Siegling, 2001).

397 Carotenoid metabolites are found in the microsomal membrane and in different subcellular
398 fractions including nuclear, mitochondrial, cytosolic, and floating lipid of the corpus luteum; in
399 particular, retinal and retinol were found in the corpus luteum of cows (O'fallon and Chew, 1984).
400 It is assumed that β -carotene in the corpus luteum tissues may be in the form of a retinol depot
401 (O'fallon and Chew, 1984). Therefore, it is metabolized during periods when the retinol necessary
402 for the corpus luteum activities is insufficient. In this context, it is interesting to observe that
403 progesterone synthesis was reduced in rats with vitamin A deficiency (Hurley and Doane, 1989).

404 The levels of retinol, retinyl esters and β -carotene in bovine follicular fluid and blood
405 plasma were reported to be closely correlated (Hidalgo et al., 2005); however, the correlation
406 between bovine plasma and corpus luteum was negligible, with the exception of retinol. This

407 molecule was closely correlated with plasma and follicular fluid in pigs, while the correlation
408 between plasma and corpus luteum retinol, retinyl esters and β -carotene was negligible (Chew et
409 al., 1984).

410 Arellano-Rodriguez et al. (2009) evaluated the effect of β -carotene supplementation on
411 luteal activity, in particular on goat progesterone synthesis. Feeding animals with 50 mg/d of β -
412 carotene for 35 days before and 17 days after ovulation, increased the synthesis and secretion
413 of progesterone by the luteal tissue.

414 Ultimately, carotenoids are important in luteal steroidogenesis and in the correct
415 succession of ovarian phases, including the luteal one. All this is also evidenced by the typical
416 yellowish color of the corpus luteum, determined by the presence of carotenoids in this endocrine
417 gland.

418 2.5. Embryos and pregnancy: beneficial effect and overdose

419 For several years, vitamin A has been associated to reproductive organs function (Eskild and
420 Hansson, 1994), embryos development and pregnancy (De Souza Mesquita et al., 2021; Quadro
421 et al., 2020; Quadro and Spiegler, 2020; Trainor, 2022). The importance of this vitamin in
422 embryonic development is demonstrated by specific offspring malformations induced by the
423 vitamin A deficiencies during gestation (Draghici et al., 2021; Gutierrez-Mazariegos et al., 2011).
424 Various studies showed the efficacy of β -carotene in increasing pregnancy rates in dairy cow
425 (Aréchiga et al., 1998) and in reducing retained placenta (Michal et al., 1994; Oliveira et al., 2015).
426 Chew et al. (1982) reported that β -carotene, has antioxidant effects, especially in the ovaries and
427 uterus of cows, enhances host defense mechanisms by lymphocyte and phagocyte, and
428 decreases mastitis. Some studies (Ozaki et al., 2017; Vermot et al., 2000; Zheng et al., 2000)
429 evidenced that RA is involved in endometrial development and renewal, being important in the
430 cyclic change during the ovarian phases and also at the time of blastocyst implantation.

431 As pregnancy progresses, uterine vitamin A concentrations decrease and this was related
432 to a supply of this vitamin by the placenta (Groothuis et al., 2002; Schweigert et al., 1999) or to
433 an increasing demand of the vitamin A by the uterus and embryo (Maden, 1994).

434 In dairy cows, deficiency of vitamin A or its natural precursor, β -carotene, resulted in
435 reduced conception rates (Hurley and Doane, 1989). The major reproductive problems in dairy
436 cows take place during late gestation, as indicated by increased rates of abortion and retained
437 placenta and the birth of dead, weak, or blind calves (Hurley and Doane, 1989). Continuous
438 feeding of β -carotene low rations reduced reproductive efficiency and had deleterious effects on
439 pituitary and ovarian function (Hurley and Doane, 1989).

440 β -carotene injections in combination with tocopherol improved the quality of embryos in
441 Holstein cows with induced superovulation (Sales et al., 2008). The addition of β -carotene to the
442 diet increased the concentration of this molecule in plasma, colostrum, and milk of mares and

443 also in the plasma of their foals (Kuhl et al., 2012). However, no positive effect on female fertility
444 was noted (Kuhl et al., 2012).

445 Pharmacological concentrations of RA lead to embryo toxicity, when administered shortly
446 after implantation (Huang et al., 2001; Piersma et al., 2017).

447 In pigs, retinol and RBP, were abundantly produced by the uterus, so there was a high presence
448 of these molecules in the uterine fluid (Schweigert et al., 1999). These studies evidenced a
449 noteworthy role of retinol and RBP on the early embryo trophism, as confirmed in the ewe (Doré
450 et al., 1994), and gilt (Schweigert et al., 1999).

451 In particular, the RBP synthesis was active in uterine glands and uterine surface epithelium
452 as demonstrated in different species: sows (Adams et al., 1981; Harney et al., 1994; Wang et al.,
453 2012), baboon (Fazleabas and Verhage, 1994), mares (McDowell et al., 1995), goat (Liu et al.,
454 1995), mouse (Ma et al., 2012), rat (Itoh et al., 2009), cow (Costello et al., 2010; Mullen et al.,
455 2012). The expression of the RBP is hormonally regulated and ovarian cycle dependent. In fact,
456 Schweigert and Siegling, (2001) described the localization of RBP, CRABPI, and RXR in the sow
457 genital organs, during different stages of the estrous cycle. In particular, the highest concentration
458 of RBP and RXR were observed during estrus. In the endometrium and myometrium, RXR was
459 present throughout the entire cycle. Its highest concentration was recorded during diestrus,
460 whereas RBP and CRABPI were found in endometrial cells only during diestrus. Moreover,
461 CRABPI expression in oviductal tissues appeared to depend on estrogen. In contrast, in the
462 uterus, RBP and CRABPI expression was influenced by progesterone (Schweigert et al., 1999).

463 In the endometrium, gene expression for RBP was sensitive to small fluctuations in
464 progesterone concentration on the 7th day of the sexual cycle. However, the authors did not find
465 a significant correlation between progesterone concentration in the blood plasma and uterine fluid
466 and that of RBP on day 7 of the cycle (Costello et al., 2010).

467 Vitamin A deficiencies found in developing countries during pregnancy lead to alterations
468 in embryogenesis (Hovdenak and Haram, 2012), however, overdose is often found in developed
469 countries, causing a teratogenic effect. Since 1954, Cohlan described the teratogenic effects of
470 an overdose of vitamin A during pregnancy in the rat (Cohlan, 1954). The teratogenic effects of
471 carotenoid intake were subsequently confirmed in other animals and in humans (Geelen and
472 Peters, 1979; Rosa, 1987). Moreover, pharmacological concentrations of RA lead to embryo
473 toxicity, when administered shortly after implantation (Huang et al., 2001; Piersma et al., 2017).

474 During pregnancy, it must not be exceeded the 5000 IU/day of retinol supplementation to
475 avoid the overdose effect (Duerbeck and Dowling, 2012).

476 3. Male

477 3.1. Impact of carotenoids on male reproductive function and spermatogenesis

478 Several studies reported that deficiencies of vitamins A, RA and retinol have been
479 correlated with degeneration of testis parenchyma and spermatogonia with consequent loss of
480 the reproductive function. In bulls, β -carotene deficiency negatively affected sperm motility and
481 induced morphological alterations of the head and cytoplasmic droplet in middle piece,
482 suggesting that these alterations were likely due to retarded spermatogenesis and spermatid
483 maturation disturbances in the epididymis (Weiss et al., 1979). In another study on dairy bulls,
484 low vitamin A diet determined not only weight loss and vision alteration, but also decreased the
485 number of mature spermatozoa in the ejaculate as well as testicular atrophy (Erb et al., 1947).
486 Vitamin A deficiency caused bad semen quality and consequent low fertility. However, as
487 observed in many studies, the loss of functional germ cells is reversible, suggesting that vitamin
488 A actively participates in molecular pathways controlling spermatogenesis. The mechanism
489 underlying this phenomenon has been clarified in mouse. When male mice are knock-out to be
490 deficient of vitamin A, terminally differentiated germ cells are not present in the seminiferous
491 epithelium, where only type A spermatogonia and Sertoli cells can be found (Hogarth and
492 Griswold, 2010). Many studies have shown that administration of RA into these knock-out male
493 mouse activate the molecular pathways controlling spermatogonial differentiation to mature
494 spermatozoa (Agrimson and Hogarth, 2016; Griswold et al., 1989; Hogarth et al., 2015; Hogarth
495 and Griswold, 2010; Van Pelt and De Rooij, 1990).

496 3.1.1. Mechanism of action of Stra8 and Kit

497 The supplementation of RA to the culture of neonatal testes and undifferentiated
498 spermatogonia was correlated with higher expression of Stra8 and receptor tyrosine kinase (Kit)
499 transcripts (Pellegrini et al., 2008; Zhou et al., 2008). These genes are established markers of
500 differentiating spermatogonia and are important in increasing the number of cells containing
501 nuclei reminiscent of leptotene and zygotene spermatocytes (Pellegrini et al., 2008). Consistent
502 with these results, in another study, the injection of RA in newborn and adult mice induced higher
503 expression of Stra8 transcript (Snyder et al., 2010; Zhou et al., 2008), once again demonstrating
504 the activity of RA to control genes related to spermatogenesis. Overall, these results support the
505 theory that RA is synthesized from retinol in situ and degraded or stored with specific enzymes in
506 testes (Hogarth et al., 2015). In particular, the concentration of RA seems to continuously change
507 in the seminiferous epithelium resulting in a cyclic content variation of these enzymes (Sugimoto
508 et al., 2012). RA level is relatively low in stages II–VI and high from stage VII spermatogonia
509 (Hogarth et al., 2015). Whereas, in stages VII–VIII spermatogonia, RA level is high (Endo et al.,
510 2015). The high concentration seems to drive the progenitor-to-A1 transition and meiosis entry,
511 simultaneously. In conclusion, it clearly appears that RA regulates different stages of
512 spermatogenesis and is involved not only in spermatogonia differentiation but also in the

513 regulation of progenitor self-renewal of spermatogonia. Among the regulated genes, Kit (Koli et
514 al., 2017) and Stra8 (Raverdeau et al., 2012) (Figure 6) are direct targets of RA. To further support
515 these findings, it has also been observed that RA can inhibit Pou5f1 (Okazawa et al., 1991) and
516 Ngn3 (Hao et al., 2016) (Figure 6) which are markers of spermatogonia progenitors. Moreover,
517 RA administration in spermatogonia inhibited the expression of Gfra1, Id4, Zbtb16, Nanos2, and
518 Nanos3 (Hao et al., 2016; Koli et al., 2017) (Figure 6). Therefore, RA has the ability to
519 simultaneously down-regulate certain self-renewal and progenitor genes and up-regulate several
520 differentiating actors. However, further studies are necessary to clarify the mechanisms of action.

521 3.1.2. Regulation of Retinoic Acid Induced 14 on spermatogenesis

522 The use of WIN 18,446 has helped explaining the mechanisms of RA signaling in
523 spermatogenesis. This molecule inhibits the conversion of retinol to RA in the postnatal testis and
524 in the embryonic gonad. This makes WIN 18,446 an excellent factor to use to continue the
525 research into how RA regulates germ cell development in testes. Until now, RA signaling has
526 been controlled by inhibiting the receptors, using gene knockout studies or receptor antagonists.
527 However, these methods can be only applied to a particular receptor expressed in a specific cell
528 type making difficult the elimination of functional redundancy that may occur between receptors.
529 Following the research on WIN 18,446, another gene developmentally regulated by RA is Retinoic
530 Acid Induced 14 (RAI14). RAI14 was originally identified in human retinal pigment epithelial cells
531 (Kutty et al., 2001). However, in humans, RAI14 is also expressed in placenta and testes (Kutty
532 et al., 2006). RAI14 protein is composed by six ankyrin repeats and a long coiled-coil domain
533 which is at the N-terminal region and at the C-terminus, respectively, which are domains involved
534 in protein-protein interactions (Kutty et al., 2006). In rat testes, RAI14 is expressed in germinative
535 epithelium, in both the Sertoli and germ cells (Qian et al., 2013a). In the same study, it was also
536 demonstrated that RAI14 localizes at both the basal and the apical ectoplasmic specialization,
537 demonstrating that RAI1 regulates F-actin organization at this level. These results were consistent
538 with those of another study, where the knock down of RAI14 in Sertoli cells *in vitro* mediated by
539 small interfering RNA in Sertoli altered cell junction functionality as well as F-actin distribution
540 (Qian et al., 2013a). Furthermore, in the same study, it was demonstrated, that the inactivation of
541 Rai14 affected spermatid polarity, adhesion and spermatid movement (Figure 6), because of the
542 disruption of the apical ectoplasmic specialization (Qian et al., 2013b). Finally, RAI14 has also
543 been found to be predominantly expressed in mouse testis (Kutty et al., 2006). However, further
544 work will be necessary to elucidate these findings as little is still known about its RAI14 function
545 during mouse spermatogenesis.

546 3.1.3. Involvement of Dmrt1 into the regulation of RA signaling pathway

547 Recently, the mechanisms underlying the role of RA signaling pathway have been clarified
548 in goat. In particular, the researchers discussed a possible role of Doublesex and mab-3 related

549 transcription factor 1 (Dmrt1) in the RA signaling pathway (Figure 6). As discussed earlier, Stra8
550 and RA are regulators of meiosis, which one of the principal mechanisms characterizing
551 spermatogenesis (Matson et al., 2010; Raverdeau et al., 2012). While, in female fetal gonads, RA
552 activates transcription of Stra8 which allows the beginning of meiosis. In the fetal male gonad,
553 these mechanisms are inhibited by Cytochrome P450 Family 26 Subfamily B Member 1
554 (CYP26B1) (Feng et al., 2014).

555 Dmrt1 has two main functions: 1) it activates spermatogonia proliferation and
556 differentiation before meiosis initiation and promotes expression of spermatogenesis and
557 oogenesis-specific basic helix–loop–helix 1 (Soxhlh1) (Matson and Zarkower, 2012); 2) it
558 coordinates mitosis and meiosis by repressing RA signaling and inhibiting Stra8 transcription
559 (Matson et al., 2010). In male gonad of dairy goat, Dmrt1 expression was significantly higher than
560 in other tissues (Wei et al., 2018). Recent evidences have shown that RA inhibits Dmrt1
561 expression with negative impact on spermatogonia differentiation (Wang et al., 2016). On the
562 contrary, overexpression of Dmrt1 *in vitro* was associated with down-regulation of Stra8 and
563 Synaptonemal Complex Protein 3 (Scp3) and enhancement of differentiation and proliferation of
564 male goat germ cells. Therefore, these results indicate that Dmrt1 exhibits a significant effect in
565 spermatogenesis and maintenance of mammalian spermatogonia (Wei et al., 2018).

566 3.1.4. Correlation of retinoic acid with gut-testis axis

567 Deficiency of RA could be also caused by altered diet and in particular there is a correlation
568 between RA absorption and gut health. In sheep, it has been proposed that the mechanisms of
569 vitamin deficiency could be due to a gut–testis axis alternations. Zhang et al. (2022) showed that
570 the use of induced excessive energy diet model altered spermatogenesis. This seems to be
571 dependent on reduced bile acid levels, which further influenced RA absorption. Overall, these
572 findings demonstrated that modification of gut microbiota and alteration of RA metabolism have
573 potential as treatments for male infertility induced by excessive-energy diet-induced metabolic
574 syndrome (Zhang et al., 2022).

575 3.2. Antioxidant effect of carotenoids

576 Carotenoids are known to exert an antioxidant effect on testicular cells thus ameliorating
577 the impact of free radicals. In rat, β -carotene decreased the negative effect of methotrexate
578 induced testicular injury thanks to the anti-oxidant and anti-apoptotic effects (Vardi et al., 2009).
579 In another study, β -carotene ameliorated the effect of ethanol on hepatic cells (Peng et al., 2010).
580 This seemed to occur through the inhibition of caspase-9 and caspase-3 expression which
581 determined an apoptotic effect on the treated animals (Peng et al., 2010), once again showing a
582 potential role of β -carotene as anti-oxidant.

583 Amongst the beneficial effects, it has been observed that carotenoids administration
584 improves critical semen parameters including sperm motility, membrane and DNA integrity since,

585 *in vivo*, they protect spermatozoa from reactive oxygen species (ROS) produced by leukocytes,
586 reduce cryodamage to spermatozoa, block premature sperm maturation and provide an overall
587 stimulation to the male gamete (Bansal and Bilaspuri, 2011; Sheweita et al., 2005; Twigg et al.,
588 1998). Moreover, an increasing number of reports are emphasizing on the beneficial antioxidant
589 role of carotenoid on frozen semen (Bucak et al., 2015). This effect increases when carotenoids
590 are used in combination with other antioxidant compounds such as curcumin (Reddy and Lokesh,
591 1994). Curcumin acts as antioxidant thanks to the phenolic groups which eliminate oxygen-
592 derived free radicals and superoxide anions (Piper et al., 1998; Reddy and Lokesh, 1994). The
593 action of curcumin on markers of oxidative stress is correlated with its properties able to determine
594 the removal of reactive oxygen and nitrogen, metal chelation, and regulation of numerous
595 enzymes. In particular, curcumin increases the activity of glutathione peroxidase (GSH-Px),
596 catalase and superoxide dismutase (SOD) enzymes that neutralize free radicals, it inhibits
597 enzymes (lipoxygenase, cyclooxygenase, xanthine oxidase) that produce ROS (Lin et al., 2000;
598 Piper et al., 1998) The use of curcumin improved spermatogenic disorders induced by scrotal
599 heat stress in mice (Lin et al., 2015). In this study, co-treatment with β -carotene or curcumin led
600 to repair activity, as indicated by the presence of many spermatogenic cells. In particular, the
601 combined treatment with β -carotene and curcumin resulted in recovery to almost normal testicular
602 morphology. Therefore, β -carotene and curcumin could be natural protective candidates to
603 protect against male infertility induced by various environmental stressors.

604 However, although many studies have demonstrated the possible use of carotenoids as
605 antioxidants, further work is necessary to clarify their mechanisms of action.

606

607 4. Conclusions

608 Carotenoids are a class of natural pigments synthesized by plants, algae, and photosynthetic
609 bacteria. Mammals are not able to synthesize carotenoids and they have to take them from the
610 diet. It is well-known that carotenoids are important in regulating tissue growth and act in
611 promoting the reproductive function of female and male. In the present work, we summarize the
612 findings on the mechanisms of action of carotenoids and its derivatives in controlling
613 folliculogenesis and oogenesis and have a steroidogenic function in the females. Carotenoids can
614 be potentially used alone or in combination with other hormones for its moderate estrogenic effect.
615 Whereas, in the males, carotenoids activate the molecular pathways related to spermatogenesis.
616 Several studies have also shown that deficiency of these vitamins can alter the processes of
617 spermatogonia development and induce infertility with consequent absence of mature
618 spermatozoa. Carotenoids have an antioxidant effect which seems to be exerted by ameliorating
619 the activity of free radicals. The mechanisms of action seem to be exerted by activating KIT and
620 STRA8 pathways in both female and male.

621 Overall, in the present review, we show that carotenoids can be potentially supplemented in
622 the animal diet to favor the reproductive function. However, although considerable research
623 supports the positive impact of carotenoids on animal reproduction, further studies are necessary
624 to consolidate the knowledge on the properties of carotenoids and their role in the reproductive
625 functions including the determination of the beneficial dose which should not be exceeded to
626 avoid a toxic effect.

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629 **Human and animal rights**

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644

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1236

1237 Figure legends

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1239

1240 Figure 1. Schematic representation of the chemical structure of β -carotene. From Saini et al.
1241 (2015).

1242

1243 Figure 2. Characteristic end groups of carotenoids (names of carotenoids written in bracket are
1244 examples of carotenoids). From Namitha and Negi (2010).

1245

1246 Figure 3. β -Carotene metabolism. Enzymatic activities in italics

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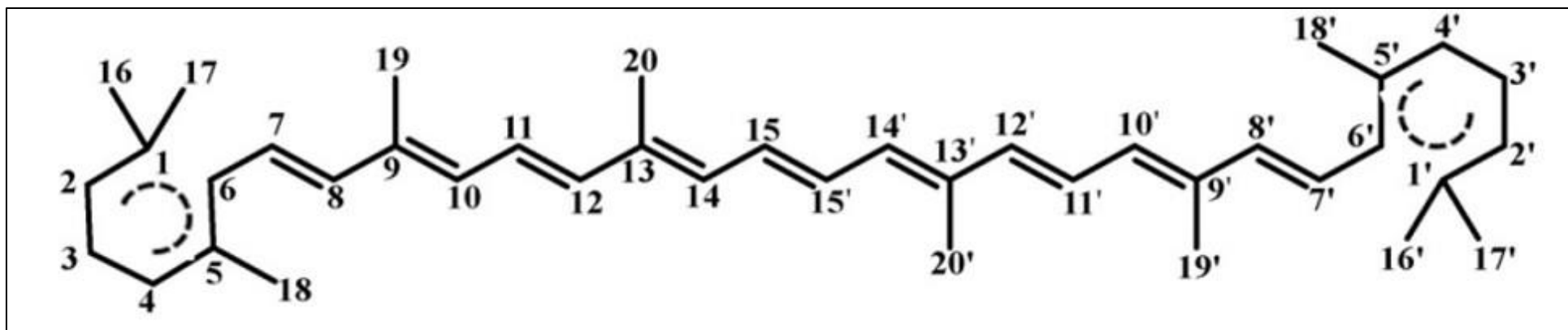
1248 Figure 4. Schematic representation of the mechanisms related to RA and retinol and (A) and β -
1249 carotene (B) on steroidogenesis

1250

1251 Figure 5. Schematic representation of RA signaling pathways involved in oogenesis.

1252

1253 Figure 6. Schematic representation of the RA mechanisms of action in the male reproduction
1254 function.



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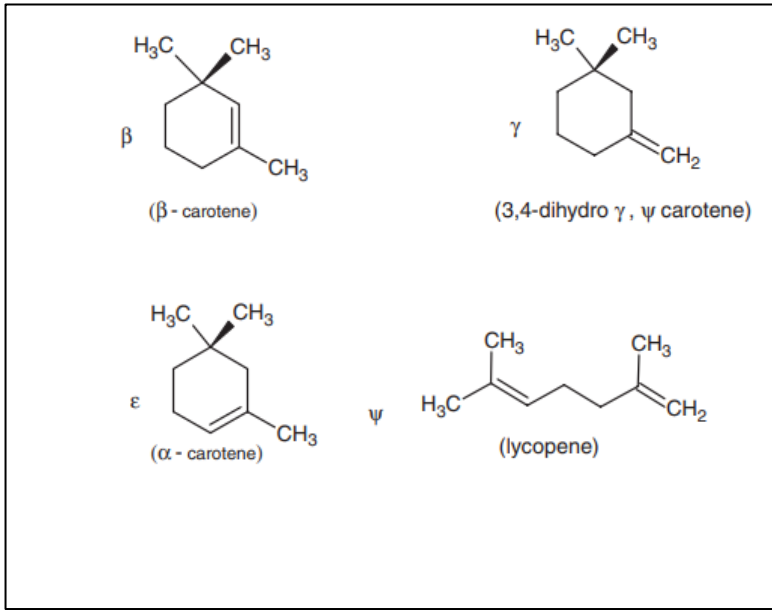
1256

1257 Figure 1

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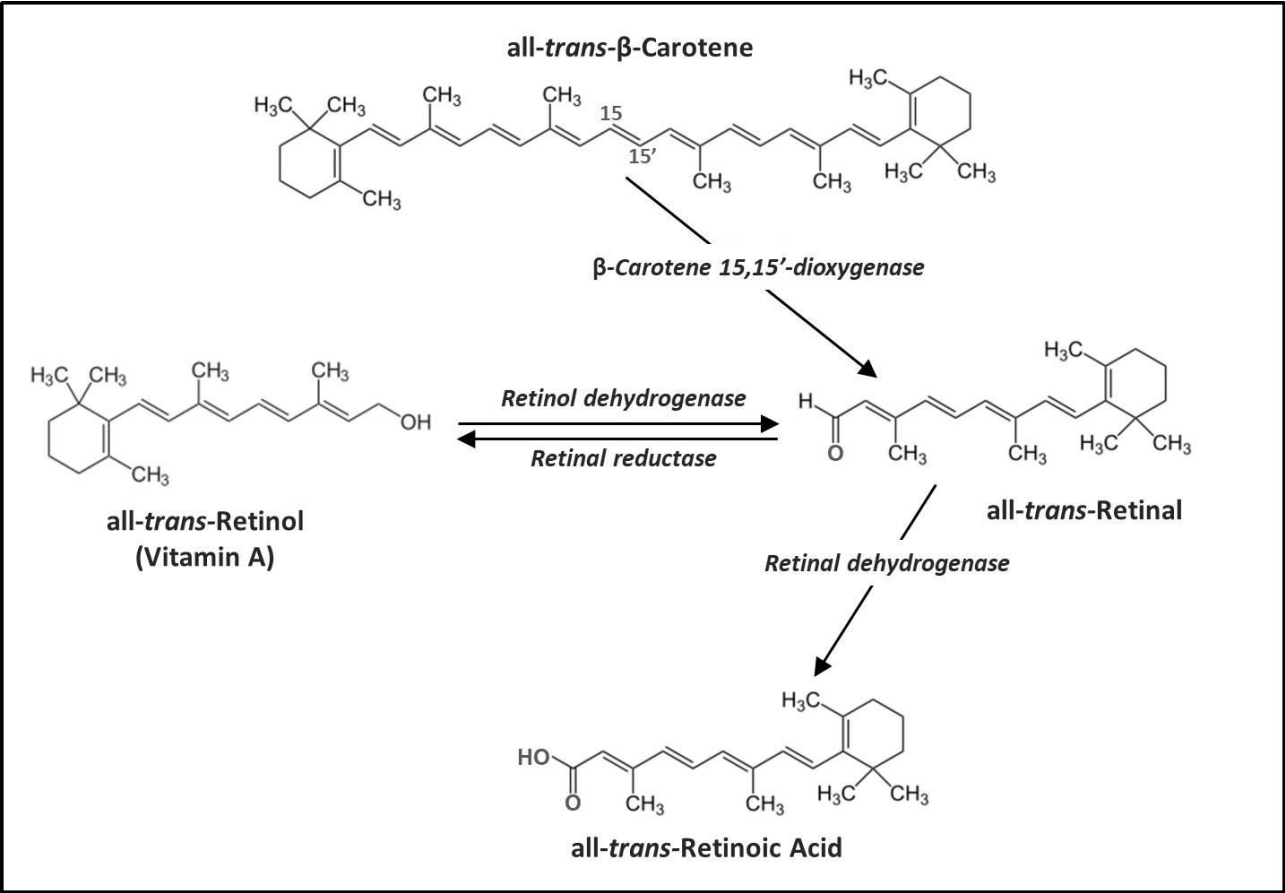


1261

1262 Figure 2

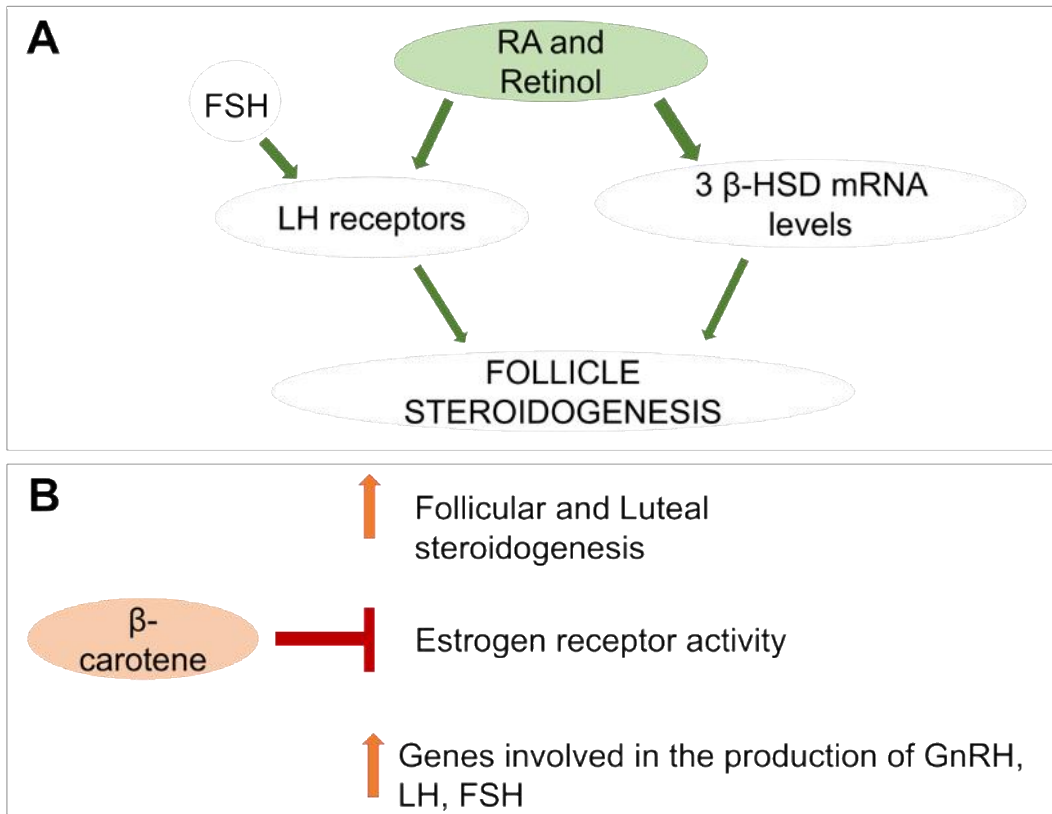
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1265
1266 Figure 3
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Steroidogenesis

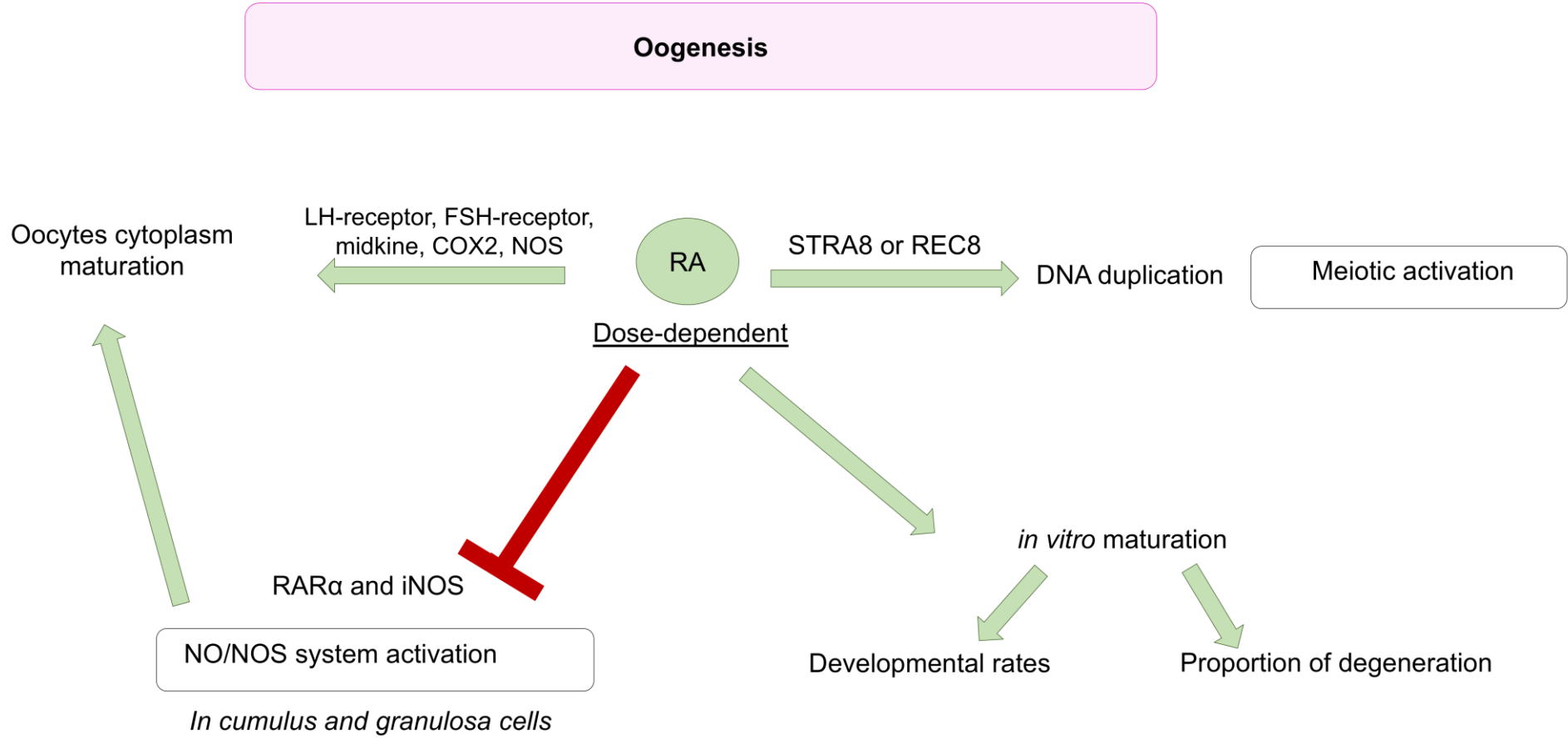


1269

1270 Figure 4

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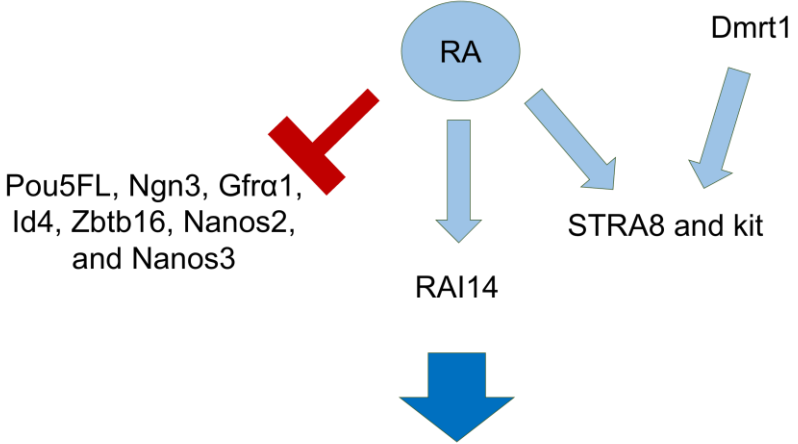
1273

1274 Figure 5

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Spermatogenesis



- ✓ Self-renewal of spermatogonia progenitor
- ✓ Differentiation of spermatogonia
- ✓ Maturation of sperm cells (polarity, motility and head speciliazion)

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Figure 6

Species	Plasma/Serum		Follicular fluid		Corpus luteum	
	Retinol	beta-carotene	Retinol	beta-carotene	Retinol	beta-carotene
Cow	0.1 to 0.6 µg/ml (Nozière et al., 2006, plasma)	1 to 16 µ g/ml (Nozière et al., 2006, plasma)	0.25 µ g/ml (De Bie et al., 2016); 0.1 µ g/ml (Chew et al., 1984)	0.21 µ g/ml (De Bie et al., 2016); 0.37 µ g/ml (Chew et al., 1984); 0.41 µ g/ml (Haliloglu et al., 2002)	0.7 µ g/g wet-weight (Chew et al., 1984)	14.2 µ /g wet-weight (Chew et al., 1984)
	0.21 µ g/ml (Aytekin and Aypak, 2011, serum)	0.20 µ g/ml (Aytekin and Aypak, 2011, serum)	---	---	---	---
Goat	0.35 µ g/ml (Yang et al., 1992, plasma)	N.D. (Yang et al., 1992, plasma; Mora et al., 2000, plasma)	---	---	---	---
	30.84 µmol/l (Mora et al., 2000, plasma)					
Mare	6.58 µ g/ml (Álvarez et al., 2015, plasma)	0.67 µ g/ml (Álvarez et al., 2015, plasma)	---	---	---	---
	0.34 µ g/ml (Anderson et al., 1995, serum)	N.D. (Brief and Chew, 1985, serum); 0.03 µ g/ml (Chew et	0.18 µ g/ml (Chew et al., 1984)	N.D. (Chew et al., 1984)	0.7 µ g/g wet-weight (Chew et al., 1984)	0.1 µ g/g wet-weight (Chew et al., 1984)
Gilt						

		al., 1984, plasma)				
Bitch	6-209 pg/dl (Pérez Alenza et al., 1998, serum)	N.D. (Chew et al., 2000, serum)	---	---	---	---
Cat	0.21-0.96 µ g/ml (Crissey et al., 2003, serum)	0.011 µ g/ml (Crissey et al., 2003, serum)	---	---	---	---

1281

1282 Table 1. Retinol and β-carotene concentration in plasma/serum, follicular fluid and corpus luteum
 1283 of mammalian.N.D.: not determined.