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ConcePTION

WP 3-Determination of drug transfer and infant drug exposure during lactation: generation of quantitative and translatable data

D3.2 Report on lactation characteristics of animal species; Selection of the animal species to be used in *in vivo* studies

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Abbreviations

FIL	Feedback inhibitor of lactation
GH	Growth hormone
LA	Lobuloalveolar
MECs	Mammary epithelial cells
NHPs	Non-human primates
PRL	Prolactin
TDLU	Terminal duct lobular unit
WP	Work package

Content

Abstract

The aim of this Task (3.1) was to perform an accurate literature scanning in order to gain knowledge regarding the lactation characteristics of the most used animal species in biomedical research settings. Resulting data used to select a potential candidate species for *in vivo* studies to evaluate drug transfer into milk during lactation with a high translatable value to the human setting. Species taken into account were the most commonly used in regulatory toxicity and included rodents (rat and mice), rabbits, dogs, (mini)pigs and non-human primates (NHPs). Reference data regarding humans were also collected and analyzed in order to highlight critical similarities and differences with the studied species. Ruminants were excluded from the search since their peculiar gastrointestinal anatomy and physiology results in a relatively lower translational value when (drug) metabolism is involved. Key topics were anatomy of the mammary gland, physiology of lactation and qualitative and quantitative composition of colostrum and mature milk. As expected, from a gross anatomy point of view, the variations between the analyzed species in terms of number of glands were very high, with only NHPs showing the same number as humans, despite differences in the number of canals. Nonetheless, having a higher number of mammary glands and teats, would allow for easier sampling procedures and higher volume specimens, without disturbing the animal in the feeding process. Regarding the physiology of lactation, no major discrepancies were identified amongst the analysed species aside from some differences in the production and overall physiology of prolactin in rodents. Aside from that, hormonal inputs and pathways were mostly conserved, with prolactin and oxytocin being pivotal. The comparison of colostrum/milk compositions between the different species was relatively hard to interpret in light of several technical and physiological issues. Indeed, a lot of the retrieved papers were old and relied on poor sample sizes and different, often outdated, analytical tools. Moreover, high inter individual variations within the same species were extremely common. Overall, smaller species showed higher differences in milk composition in comparison to humans. Additional practical considerations were also taken into account, such as ethical consideration regarding the chosen species which affects the group size, financial implications and technical feasibility of lactation trials (e.g., ease of sampling, volume of sampling, husbandry requirements and scientific recognition). In conclusion, the present analysis of the literature confirmed the complexity of the decisional process behind the choice of an animal model for *in vivo* trials. For some of the evaluated species, data was either poor or missing, highlighting the necessity to generate more physiological background studies for species that are routinely used in laboratory settings. Overall, when taking into consideration ethical factors, feasible group size, milk volume and ease of milk collection, and physiological similarities with humans, the Göttingen Minipig seems to represent the most appropriate choice.

Methods

A specific aim of the IMI ConcePTION work package (WP)3 was the collection of quantitative data in an animal model regarding the potential passage of pharmacological compounds and their metabolites into milk. Thus, literature searches were performed to identify a candidate non-clinical species relevant to human for use in lactation study(ies) and experimental trials. Species taken into account were the most commonly used in regulatory toxicology and included rodents (rat and mice), rabbits, dogs, (mini)pigs and NHPs. Reference data regarding humans were also collected and analysed in order to highlight critical similarities and differences with the studied species. Ruminants were excluded from the search since their peculiar gastrointestinal anatomy and physiology results in a relatively lower translational value when (drug) metabolism is involved. This would indeed create strong biases when considering the overall aim of the WP. The topic of the hereby presented report was divided into three main critical subcategories for the assessment of lactation characteristics:

- anatomy of the mammary gland
- physiology of lactation
- colostrum/milk qualitative and quantitative composition

A preliminary scanning of the literature highlighted a relative lack of relevant data in the most used databases such as PubMED and EMBASE, making a systematic approach unfeasible. Indeed, basic data regarding anatomy and physiology are often found in textbooks or old papers and used only as reference/control data in more recent studies. Therefore, it was decided to broaden the literature search to currently used Veterinary Medicine textbooks and to use less-specific search words and their combination for the different subcategories. Further literature searches were then performed starting from the reference sections of the retrieved articles. The search criteria are provided below:

- Anatomy of the mammary gland: preliminary search words included (“mammary gland” OR “udder”) AND (“anatomy” OR “morphology” OR “structure”) AND the different species afore mentioned and their synonyms.
- Physiology of lactation: preliminary search words included (“lactation” OR “milk production” OR “colostrum production”) AND (“physiology”) AND the different species afore mentioned and their synonyms.
- Colostrum/milk composition: preliminary search words included (“colostrum” OR “milk”) AND (“composition” OR “components” OR “quantitative composition”) AND the different species afore mentioned and their synonyms.

Retrieved articles, book chapters and books were then analysed to identify potential biases to the reported results as methodological errors or experimental design errors.

Results

Anatomy of the mammary gland

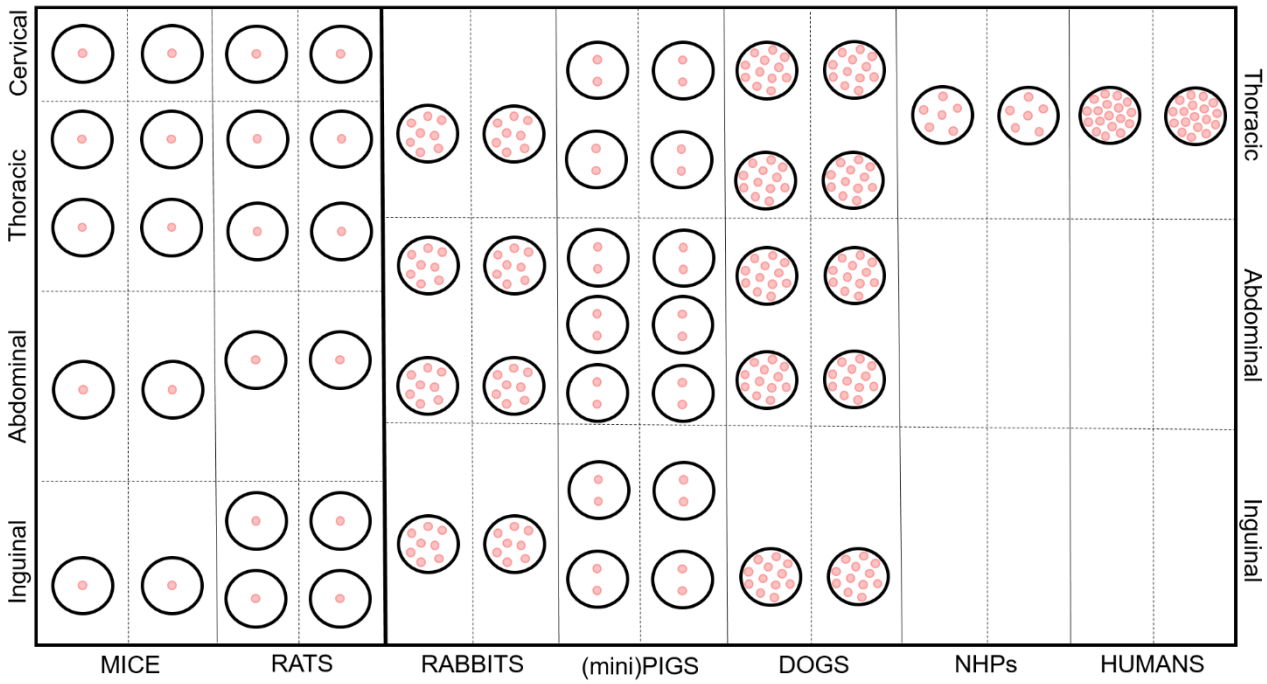
The results of the literature search regarding the anatomy of the mammary gland in the species taken into account for the present report are summarized in **Table 1**.

In general, mammary glands can be defined as modified glands that give name to the Mammal class, whose secretion is vital for the offspring survival. They are complex tubule-alveolar glands made of secretory units organized into lobules, surrounded by connective tissue septa [1]. From a developmental point of view, they originate as epithelial buds growing into the mesenchyme starting from linear ectodermal thickenings, also known as mammary ridges, and subsequently placodes [2]. Mesenchyme starts proliferating around such buds to create a teat/nipple on the skin surface. At this stage, epidermal sprouts start developing, from the buds to the teat/nipple, creating canals. Every canal will then create a separate duct that associates with a glandular mass and a separate orifice [1]. The number of overall glands, teats/nipples and canals vary amongst mammals as shown in **Table 1**, as well as the anatomical location of the mammary unit, represented in **Figure 1**. The evolution of the reproductive strategy torwards a lower number of newborns for pregnancy, often accompanied by a higher level of maternal care, is the main reason for the large difference between NHP/Human and the other species.

Table 1. Anatomical features of the udders of the species taken into account.

Species	n° of glands	n° of teats/nipples	n° of canals per gland	Location	References
Rats	12	12	1	cervical (1pr) thoracic/pectoral (2pr) abdominal (1pr) inguinal (2pr)	[3, 4]
Mice	10	10	1	cervical (1pr) thoracic/pectoral (2pr) abdominal (1pr) inguinal (1pr)	[3, 5–8]
Rabbits	8-10	8-10	6-10	thoracic (1pr) abdominal (2pr) inguinal (1pr)	[9, 10]
Dogs	8-10	8-10	7-16	thoracic (2pr) abdominal/inguinal (2-3pr)	[8, 11]
(mini)Pigs	10-18	12-18	1-3	thoracic (2pr) abdominal (3pr) inguinal (2pr)	[12, 13]
NHPs	2	2	5-7	pectoral (1pr)	[14]
Humans	2	2	10-25	pectoral (1pr)	[3, 15]

Figure 1. Schematic representation of the anatomical features and distribution of the mammary gland in the analyzed species. Pink dots represent the canals/ducts. Artwork by Alberto Elmi.



In the analysed non-clinical species, at birth the gland is just a rudimentary ductal system, that will continue to evolve and grow during puberty and, mainly, first pregnancy under the influence of a wide variety of hormonal factors [2]. Indeed, out of the vast array of mammals' tissues, the mammary gland is one of the few undergoing multiple growth, functional development, and regression episodes in the lifespan. With the post-pubertal development, the area of the gland occupied by epithelium increases, with a relative decrease in its stromal component. Such phenomenon becomes even more evident in the late stages of gestation, when alveoli grow, even if, usually, true alveoli are not formed until conception. It still has to be acknowledged that the majority of critical changes (up to 94% of the overall modifications) occur during pregnancy [16]. During gestation, vascularization dramatically increases and, by mid-pregnancy, each alveolus is surrounded by a basket-like network of capillaries [17]. Milk secretion is achieved by the afore-mentioned alveoli, formed by a single layer of secretory epithelium bound by tight junctions and arranged in a cylindrical manner. Lobules, represented by multiple alveoli surrounded by connective tissue septa, generate lobes upon further bundling [18]. Another important component of the mammary gland is represented by myoepithelial cells, responsible for milk letdown, from alveoli down to the duct, and eventually milk release [18].

Humans

Humans have a single pair of mammary glands, called breasts, positioned over the *pectoralis major* muscle of the anterior chest. In humans, the mammary tissue is divided into 15-20 lobes of parenchyma separated from each other by a highly variable amount of adipose tissue. Each lobe is drained by its own major lactiferous duct leading to the nipple. The main ducts dilate into small sinuses as they get close to the *areolus*, where they open directly on the nipple. There are about 11 to 48 minor ducts. Surrounding the parenchymal structures are fibrous thickenings of connective tissue, which connect the deep fascia with the dermis of the overlying skin to form a suspensory ligament called Cooper's ligament. The functional Terminal Duct Lobular Unit (TDLU) appears in human breasts upon sexual maturity. According to the area occupied, number of acini, secretory morphology and cellularity, Lobular Units are classified as Types 1–4, with Type 1 being the least mature and Type 4 lobules being terminally differentiated, milk producing units found in the lactating mammary gland [3].

NHPs

Non-human primates, like humans, have two pectoral mammary glands. The non-lactating mammary gland is macroscopically flattened, but the histologic appearance is nearly identical to human breasts. In macaques, as in human women, the mammary tissue lies above and lateral to the nipple, extending to the axilla. The mature gland consists of an arboreous-like ductal system and TDLUs, which are formed of a terminal intralobular duct and surrounding alveoli, embraced by myoepithelial cells. In the

non-lactating breast, only approximately 5% of the organ is occupied by glandular epithelial tissue, while the remaining 95% consists of fat, fibrous connective tissue, and vascular and nervous structures. In NHPs, each nipple is crossed by five to seven lactiferous ducts, with varying degrees of communication between the corresponding ductal and lobular units. There are occasional small clusters of glandular tissue in the nipple [14].

(mini)Pigs

Out of the analysed species, the pig shows the highest variability in the number of mammary glands, mainly imputable by the wide range of breeds spread throughout the world. Breeds with higher number of teats have been selected by the farm industry as capable of nursing larger litter sizes, with higher economical profits. Generally speaking, pigs have six/seven pairs of mammary glands, located between the thoracic (two pairs), the abdominal (three pairs) and the inguinal (two pairs) area. Each nipple has two ducts which separately overlook on two external openings [18]. At birth, each mammary gland of the piglet is composed of the teat including its thick connective tissue base, an organized fat pad of adipose lobules and connective tissue, two lactiferous ducts, and a few ducts branching into the fat pad. These structures continue to grow until puberty. A significant increase in TDLU development occurs during pregnancy, particularly after day 75; during this period, parenchymal tissue mass increases by over 200%, while parenchymal lipid decreases by nearly 70% [19].

Dogs

Regarding the canine species, mammary glands are arranged into two lines along the ventral surface as for the other species, with two thoracic, one abdominal and two inguinal pairs. However, the number of the mammary glands in dogs can vary. Indeed, sometimes, the abdominal one is missing and occasionally there are more than five pairs. The adult mammary tissue is unevenly divided: the caudal glands are larger and the tissue of the two most caudal glands is usually continuous [11]. The amount of mammary and adipose tissue present is very variable and is more abundant in the abdominal and inguinal glands than in the thoracic glands. Each teat has between 7 and 16 duct openings, and each of these ducts will eventually form a lobe of the adult gland. The larger lactiferous ducts open into the mammary sinus that is lined by a double layer columnar epithelium, whereas the smaller ducts have a single layer of cuboidal epithelium and fusiform myoepithelial cells. Every duct is surrounded by fusiform myoepithelial cells. The alveolus is composed by secretory cells, which vary from cuboidal to columnar and have variable numbers of intracellular fat droplets that accumulate in the alveolar lumina. Surrounding the alveolus in a basket-like fashion, are star-shaped myoepithelial cells. The epithelial component of the mammary gland is supported by mesenchymal tissue; this

includes fibrous connective tissue, adipose tissue, blood vessels, nerves, and lymphatics. The fibrous connective tissue may be subdivided into 2 components: the intralobular component that surrounds the intralobular ducts and the interlobular component that separates the lobules. The first one consists of finer collagen fibers surrounded by a more extensive extracellular matrix, while the second has larger collagen fibers with less extracellular matrix [8].

Mice

Mice have five pairs of bilaterally symmetrical mammary glands, located along the ventral milk line between the cervical and inguinal area. Such lines can be divided into the cervical-thoracic area, containing three glands on each side, and the abdominal-inguinal region, with two glands on each side. Each gland terminates into a single collecting duct that releases milk through a single teat. Mice mammary glands, just like rats and rodents in general, do not have separate lobe and are made of a single complex arboreous system. Each rodent mammary gland contains 5-10 secondary collecting ducts, which drain into a single lactiferous duct in the nipple [3]. The mature secretory glandular unit is lobuloalveolar (LA), which undergoes complete maturation only during pregnancy and does not normally persist following weaning. Before pregnancy, ducts have blunt ends and will only develop terminal-end buds in pregnancy. The murine ductal system is primarily surrounded by adipose tissue, with poor fibrous tissue. Rodents mammary tissue comprehends two major epithelial cell types: basal myoepithelial cells and luminal epithelial cells. Luminal cells range from tall columnar cells in the major ducts to cuboidal cells in the smaller terminal ducts and lobules. Lobular luminal epithelial cells represent the actual functional secretory cell type during lactation [5].

Rats

Rats have twelve mammary glands, distributed in six pairs along the milk line, with one pair located in the cervical, two in the thoracic, one in abdominal and two in the inguinal regions. The organization of major lactiferous ducts is similar to the mouse, with a single duct leading to the nipple's ostia [3]. The mammary glands of females, comprised of scattered tubular ducts and alveolar structures, are characterized as tubuloalveolar. There are larger, more contiguous, lobular groups of cells distinguishable for their lack of tubular/ductal orientation [20]. The mammary gland has a compound of branching tubular ducts which terminate in secretory glandular alveoli, also called acini. Lobules are composed of groups of alveoli. As for the rat and the human species, the basic milk producing unit is the TDLU, composed of a lobule associated with intralobular and extralobular terminal ducts [3].

Rabbits

When compared to the other analysed species, data regarding the anatomy of the mammary gland in rabbits was lacking and very outdated. The number of mammary glands in this species can vary from 8 to 10 depending on the genetics of the animals [10]. They are distributed from the ventral thoracic to the inguinal regions: two pairs thoracic, two pairs abdominal and one pair inguinal. Each nipple has about 8-10 ostia [18].

Physiology of lactation

Lactation can be defined as the process that combines milk secretion and its removal and represents the final stage of the reproductive cycle. In order for it to be successful, 3 pivotal events have to occur: proliferation of alveolar epithelial cells, their structural and biochemical differentiation and, finally, synthesis and secretion of milk [18]. The process that leads to milk production is also known as lactogenesis and is critically linked to the acquisition of secretory capabilities by mammary alveolar cells. It is commonly divided into lactogenesis I and lactogenesis II [21]. During lactogenesis I, mammary epithelial cells (MECs) undergo morphological differentiation and become competent to produce and secrete some milk components referred to as colostrum [22]. In such phase, production of milk components seems to be restricted to a limited number of alveolar MEC as some secretory mechanisms are still incomplete [21]. During these late phases of pregnancy, milk production is blocked by the high levels of estrogens and, most importantly, progesterone, a steroid hormone also known as the “pregnancy hormone” since all mammals rely on this hormone to maintain pregnancy. Despite differences amongst species in progesterone production during pregnancy, a drastic drop in its production at parturition is always present [23]. Such drop allows for the initiation of the lactogenic complex activation and milk production, also referred to as lactogenesis II [18]. Indeed, high levels of progesterone are capable of inhibiting the most pivotal hormone related to lactation: prolactin (PRL). Its circulating concentrations slowly increase during pregnancy so that, by the end of gestation, levels are up to 20 times over pre-pregnancy reference values. Upon clearance of progesterone and estrogens at parturition, PRL can start promoting transcription of casein mRNA, stimulating the synthesis of α-lactalbumin, and increasing lipoprotein lipase activity in the mammary gland [24]. It is extremely important to acknowledge that PRL production, distribution, and its physiological functions are quite different in rodents when compared to humans and other mammals [25]. Once initiated, milk secretion continues but with variable rates over time [18], as is referred to as lactogenesis III [26]. Removal of the milk from the mammary gland is indeed necessary to maintain production and secretion. The overall control of milk secretion requires a strong interaction between both physical and chemical factors. Concerning physical factors, the most important one is the pressure exerted from the milk present in the alveoli that leads to an inverse relationship between milk production and

intra-mammary pressure. Indeed, as milk builds up within the mammary gland, crucial supporting structures such as blood vessels are displaced resulting in poor delivery of nutrients to the alveolar cells. Once milk is removed from the gland, pressure drops, and then slowly starts building up again as new milk is produced [18]. On the other hand, chemical control of milk production occurs locally by means of an autocrine protein fraction produced by MEC known as Feedback Inhibitor of Lactation (FIL) [21]. As of today, the exact mechanism of action of FIL is not completely clear, but it seems to be capable of slowing down milk production by suppressing key factors, stimulating intracellular breakdown of casein, reducing the number of PRL receptors, and inhibiting MEC differentiation [18]. Finally, another key hormonal factor in the lactation process is oxytocin. Suckling or manual stimulation of the teat is locally detected, and the stimulus is transmitted by sensory afferents to the hypothalamus, which then initiates oxytocin release from the neurohypophysis. This hormone stimulates the myoepithelial cells that surround the alveoli to contract and cause milk to flow from the alveoli through the duct system to the teat end [27].

The physiology of lactation does not differ drastically amongst species, but the duration and the yield of both colostrum and milk are highly variable (**Table 2**).

Table 2. Duration and yield of colostrum and milk production.

Species	Duration of colostrum production	Yield of colostrum	Duration of milk production	Yield of milk	Reference
Rats	/	/	~ 21 days	/	[28]
Mice	/	/	~ 18 days	0.1-0.5 ml	[29]
Rabbits	/	/	4-5 weeks	100-200 gr/day	[30, 31]
Dogs	48 h	270 ml/day	~ 8-10 weeks	~1000 ml/day	[32–35]
(mini)Pigs	24 h	~3.75k g/day*	~ 8 weeks	4500-5700 gr/day*	[36, 37]
NHPs	/	/	~ 12 months	/	[14, 38]
Humans	96 h	~500 ml/day	~ 6 months	~800 ml/day	[21, 39]

/ = data not available
* = these data only refer to standard pigs

Humans

Oxytocin, that slowly increases during late gestation and peaks at parturition, triggers milk ejection by inducing the contraction of myoepithelial cells and possibly by direct effects on the secretory activity of MEC; even bonding and maternal behaviors are regulated by oxytocin. In women, little to no milk can be obtained without activation of the milk ejection reflex (activation of both oxytocin and prolactin release). To be consistent with the duration of lactation in other primates, the average duration of

lactation in primitive women would be expected to be about 3 – 4 years [40], but nowadays the duration of breast-feeding in traditional highly industrialized societies varies greatly. It is impossible to determine 'normal' weaning behavior for both women and other mammals because the artificial termination of the lactation period is based either on social and cultural 'acceptability' or economic expediency [30].

NHPs

Non-human primate species have a high degree of similarities with humans. Oxytocin is low during the 3rd trimester of pregnancy, peaks on the date of parturition and returns to baseline levels during lactation [38]. Prolactin is not an obligate component of mammary growth and development in macaques but is required for lactation; this hormone is not as strong of a mitogen in the NHPs breast as steroid hormones or growth hormone (GH). In both human and non-human primates, the hepatic and intra-mammary enzymatic systems are present for conversion of precursor to a more bioactive estradiol (aromatase and steroid sulfatases); thus, the amount of local estrogen exposure in the breast correlates only weakly with the serum concentration. Gestation in macaques is approximately 150 days in length, and during this time, the breast, as in other mammals, undergoes extensive growth and differentiation under the influence of high systemic concentrations of estrogens, progestogens, chorionic gonadotropin, placental lactogen, and prolactin. The change in volume of the glandular tissue is roughly ten-fold to twenty-fold, as a result of both epithelial proliferation and secretory distention of the ductal and alveolar system [14].

(mini)Pigs

The peripartum prolactin (PRL) surge begins about 2 days prepartum and extends through several days postpartum, although it remains significantly greater than those found during most of pregnancy. The prepartum peak of PRL is essential for the onset of lactation and the decline slowly begins over the initial days postpartum [30]. In pigs and minipigs, the hormone relaxin from the corpora lutea has a similar function to placental lactogen, produced in humans and rodents, which is a prolactin agonist [23, 41]. Regarding the porcine species, most of the studies concerning lactation have been carried out under intensive breeding conditions and there is little information about pigs' lactation in wildlife. Wild sows build a nest for their litters and the piglets remain in the nests for about 2 weeks and are weaned after an 8-week lactation. In commercial intensive piggeries, the length of lactation has been truncated to 21-28 days (3-4 weeks), to increase profitability. The important role of milk ejection in lactation is clearly illustrated by the characteristic behavior pattern associated with suckling and the oxytocin release in the domestic sow, resembling what happens in humans. The piglets jostling and nuzzling on the mammary glands induce oxytocin release, followed by rapid ejection of milk from the

mammary glands. Piglets have a very short amount of time, in terms of minute, to obtain all the possible milk from their preferred nipple [30].

Dogs

Dogs also show a similar physiology of lactation with the drop in progesterone and estrogen post parturition, and increased levels of prolactin from mid gestation to weaning with higher spikes starting at parturition. Placental relaxin secretion starts from mid gestation, decreases in third trimester, and drops at parturition [42].

Mice

Although progesterone signalling controls alveolar proliferation, prolactin directly controls epithelial cell differentiation [43]. Its release is essential for the proliferation and functional differentiation of lobulo-alveolar structures during pregnancy [44]. Moreover, as opposed to humans, PRL has a strong luteotropic action in rodents, promoting progesterone production during pregnancy [25]. The estrogen hormone has receptors in both stromal and epithelial cells, but it is required only in the stroma for proper ductal development. Oxytocin, when released, induces the contraction of the myoepithelial cells surrounding the alveoli and thereby induces milk ejection. Thus, oxytocin is not only necessary for postpartum milk ejection but also for alveolar cell proliferation [43].

Rats

The physiology of rat lactation is similar to that reported for the mouse. In rats' mammary gland, PRLR expression is low during most of pregnancy and starts increasing on day 21, potentially in response to the pre-partum rise in pituitary PRL release, and continues to increase throughout lactation [45]. In female rats, lactation induces the mobilization of fat stores and a large increase in food intake, depending on the size of the suckling litter. In rats, prolactin secretion is suppressed during the second half of pregnancy [46]. The placenta produces the placental lactogen, which binds to prolactin receptors and stimulates growth and differentiation of epithelial cells in the udder in the same way as prolactin [41].

Rabbits

Data regarding the physiology of lactation in rabbits is unfortunately quite poor and outdated. What is known is that lactation usually lasts up to 4- 5 weeks [30], and that the litter only suckles one or twice a day. Also in this species, the key factor for lactation and its maintenance is prolactin [31].

Colostrum and milk composition

Milk and colostrum composition vary greatly among animal species. Components of milk and colostrum include proteins, lipids, carbohydrates, minerals, vitamins, and cells. The milk components were found to be influenced considerably by the stage of lactation, where these changes differ often from one species to another. In general, colostrum differentiates from milk mainly due to its high immunoglobulins (IgG) concentration. It is important to acknowledge that the different types of placentation and active immunity transport mechanisms, peculiar to each species, highly influence the degree of colostrum-mediated transfer of immunity. The degree and timing of immunity transfer during pregnancy to the offspring, impacts on the importance of colostrum for immunity transfer [18, 47]. In humans and NHPs, IgG are transferred to the fetus during the second and the third trimester of pregnancy while in rodents and rabbits IgG are transferred to the fetus mainly during organogenesis [47, 48]. In human and NHP most of the IgG are transferred to the fetus during pregnancy while in other mammalian species, such as the dog and the pig, IgG transfer during pregnancy is minimal hence the immunity transfer from the dam to the offspring is essentially lactogenic, with 85-95% of the blood immunoglobulins originating from colostrum transfer [49, 50]. Overall, IgG colostrum concentration is specifically high after parturition and rapidly drops. In addition to systemic immune protection, colostrum also plays a major role for local digestive system due to the presence of IgA, isoenzymes, lactoferrin, white blood cells and various cytokines. The newborn absorbs colostrum IgG from the digestive tract into the blood stream. The newborn ability to absorb IgG ends shortly after parturition [32]. While colostrum has higher concentrations of immunoglobulins in all mammalian species, the concentration of other components may vary between species. Very few papers investigating the milk composition early in lactation were found in our search. Here are shown the main characteristics and differences of the mammary secretion of the species examined for colostrum (**Table 3**) and “mature” milk (**Table 4**), respectively.

Table 3. Colostrum composition

Species	Dry matter %	Protein %	Casein %	Whey protein %	Fat %	Lactose %	Fe µg/ml	Cu µg/ml	Zn µg/ml	Mn µg/ml	Mg µg/ml	Ca µg/ml	P µg/ml	References
Rats	/	8.6-9.1	/	/	13.6-15.7	2.3-2.6	8.1-9.2	8.6-9.8	13.3-14.2	0.3-0.4	168-180	755-829	/	[51]
Mice	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Rabbits	31.4-33.7	13.5-15.9	/	/	13.7-20.4	1.6-2.1	/	/	/	/	/	/	/	[52]
Dogs	/	12.4-16.2	60.7	39.3	13.2	1.7	3.7	1.3	5	/	128	1363	935	[53]
(mini)Pigs	20.1-26.7	7.7-16.6	1.5-3.4	7.9-14.8	6.4-8	2.8-3.9	/	/	/	/	100	800	1000	[37, 54]
NHPs	/	2.2-2.7	/	/	4.3-6.3	7.7-7.9	0.9-2.6	2-4.1	3.5-6.8	/	37.5-61.7	324-347	/	[55]
Humans	11.92	2.6	0.4	1.18	3	5.8	1.1	0.4	4.8	0.01	32	293	159	[56–58]

Data are expressed as ranges or single values.
/ = data not available

Table 4. Mature milk composition

Species	Dry matter %	Protein %	Casein %	Whey protein %	Fat %	Lactose %	Fe µg/ml	Cu µg/ml	Zn µg/ml	Mn µg/ml	Mg µg/ml	Ca µg/ml	P µg/ml	References
Rats	27.9-32.8	8.9-9.7	6.4-8	0.9-2.5	14-15.9	1.1-4.1	4-7	1.7-7	20333	/	158-192	2849-3206	1600-2720	[59, 60]
Mice	36.3-39.4	10.1-12.7	/	/	19.3-22.9	2.4-2.8	/	/	/	/	/	/	/	[29]
Rabbits	31.2	10.3	/	/	15.2	1.8	0.003§	0.002§	0.02-0.03§	0.0001§	0.35-0.45§	2.71-5.36§	2.44-3.28§	[52, 61]
Dogs	22.7-26	4.3-9.8	65.8-75.4*	26.4-34.2*	2.4-13.4	29.3-40.2	1.8-13.1	0.9-2	4.1-9.6	0.1-0.2	55.8-104.3	1366-2440	914-1401	[33, 53, 61–63]
(mini)Pigs	18.8-22.7	5-7.5	2.7-3.6	2.4-5.4	7-10.1	4.3-5.6	/	/	/	/	105	2000	1420	[37, 54]
NHPs	12.2-14	1.3-2.3	45*	55*	3.3-6.2	4.8-9.1	/	/	/	/	34	380	152	[61, 64–67]
Humans	12.6	1.2	0.3	0.7	4.1	7	0.5-1.8	0.2-5.2	0.7-3.8	0.01-0.03	25-33	230-310	130-190	[68–70]

Data are expressed as ranges or single values.
/ = data not available
* = % on total proteins
§ = these data are expressed in g/kg

Humans

As for the other mammals, the composition of human milk is affected by different factors and, depending on the individual, changes over the course of a single breast-feeding session, of a day, and through lactation [57, 69]. One of the key factors seems to be represented by the maternal diet: indeed dietary intake of different nutrients, particularly fatty acids and some micronutrients, is related to their content in breast milk composition, but does not affect macro-nutrient composition [71]. Generally speaking, fat concentrations tend to increase not only over the course of a day, but also during the same breastfeeding session [69]. On the other hand, the overall protein and amino acids contents show a marked decreasing trend over time during the first year of lactation [57]. One of the few components that seems to remain relatively stable for the entire duration of lactation, exception made for colostrum, is lactose [57]. Regarding minerals, magnesium, phosphorus and calcium show significant lactation-stage-specific differences and high inter-individual variations, while manganese, copper and iron remain relatively constant during all lactation stages. Zinc concentrations, overall, show a decreasing trend as lactation goes on [57].

NHPs

Non-human primate milk is relatively diluted: it generally consists of <15% dry matter, with about 7% sugar, ≅3–6% fat, and ≅1–2% proteins, and changes over the course of lactation. It is important to acknowledged that, amongst different NHPs species, great differences in milk composition can be observed, mainly imputable to length of lactation, frequency of feeding and milk yield [72, 73]. Most papers investigated the NHP milk composition used the rhesus macaque, while very few papers investigating the milk composition of cynomolgus monkeys, which is the most common NHP in research, were found. In rhesus macaque (*Macaca mulatta*) both fat and protein contents increase as infants age, in the light of the higher demand in energy. Such increase in energy content seems to be related to lower milk yields. Out of the different components, fat percentages show the highest inter individual variation within the same species, while lactose levels are relatively stable [66]. However, differences in milk composition among prosimians may be related to differences in maternal care: species that carry their offspring produce more dilute milk, with higher yields, when compared to species that usually leave newborns for prolonged period. Lorises, bushbabies, and potentially cheirogaleids produce relatively rich, energy-dense milks in comparison with anthropoid primates, such as rhesus macaques (*Macaca mulatta*), white handed gibbon (*Hylobates lar*) and gorilla (*Gorilla gorilla gorilla*) [72].

(mini)Pigs

When compared to mature milk, colostrum has higher concentrations of protein, particularly

immunoglobulins, some minerals (particularly copper, iron, iodine, and zinc) and vitamins, hormones and growth factors. Lactose is present in lower concentrations in colostrum than in mature milk. Milk fat concentration transiently increases during the period from day 2 to day 4. The composition of milk after approximately day 7 to day 10 is relatively stable for the remainder of lactation. As for other species, maternal diet can affect some milk components, including concentrations of fat, fat-soluble vitamins and some minerals, as well as proportions of specific fatty acids. Some components of sow milk also are affected by genetics, parity, colostrum and milk yield, and ambient temperature [37].

Dogs

Early studies showed the dog milk composition might change with breed. For this summary, we focused on the beagle dog as it is the commonly used breed for research purposes.

To summarize, the concentration of iron, zinc, calcium, protein and fat showed patterns that were influenced by the stage of lactation. The concentration of copper, manganese, magnesium and carbohydrates were not significantly affected.

Adkins et al found that protein concentration was high in colostrum, decreased significantly by Day 21, and then slighty increased throughout the duration of lactation. This pattern of decrease protein concentration during lactation is similar to humans [53]. However, Lonnerdal et al reported that protein concentration increased in time [62]. Concentration of amino acid content were similar to those in humans. A decrease pattern in the concentration of all amino acids with increasing lactation stage was observed, similar to humans. The lipid content does not show remarkable changes during the lactation period. Slight non-significant decreases were observed between days 14 to 28. The lipid concentration in dog milk was higher than that reported in humans [53]. Lactose levels were low in colostrum, increasing gradually until Day 28, followed by a slight decrease. Iron concentration increased significantly from Day 1 to Day 3, then gradually decreased by Day 42. This is in contrast to humans where iron concentration is high in the colostrum and then gradually decrease during lactation [53]. Lonnerdal et al reported that Zinc concentration decreased throughout lactation [62] while Adkins et al reported that zinc concentration slowly increased from Day 1 to Day 14 and it then decreased by Day 42. Zinc levels were higher than those reported for humans [53, 62]. Copper concentration was slightly higher in early lactation and then gradually decreased throughout lactation or remained unchanged. Copper levels were generally higher than reported in humans. Milk calcium concentration was lower in colostrum but increased thereafter peaking on Day 35. Calcium levels were significantly higher than reported for humans [53, 62]. Magnesium concentration was highest in colostrum but rapidly decreased by Day 3 and remained relatively constant during lactation. Concentration of phosphorus showed very mild increase from Day 3 to Day 28 [53]. The iron concentration in the dog milk are influenced by the stage of lactation, with values decreasing in time. This is similar to what was reported in other species however, the iron concentration in dog milk was

found to be considerably higher than that of human milk. The manganese concentration was not found to be influenced by the stage of lactations and its levels were higher than reported for human milk. The fat content of canine milk was found to be influenced by the stage of lactation, with concentrations increasing during the first part of lactation and decreasing during the last part [62]. The level of carbohydrates was fairly constant and did not show a strong developmental pattern.

Mice

Very few papers were published on mouse milk composition. The composition of mouse milk can vary considerably between mouse strains. In general, the analysis on mouse milk is challenging due to the small sample volume and the high fat content. Crude protein levels did not change during lactation [29, 74, 75]. Crude fat increased from Day 3 of lactation to Day 14 and remain stable till Day 18 [29, 74], while in another study the fat content decreased from early to mid-lactation [76]. Lactose content increased with lactation day [29, 75]. Great variability was noted in lactose content and crude protein between strains of mice.

Rats

There are characteristic differences in the nutrient content of milk among strains of rats, particularly for changes in the lactose and fat content of the milk during the lactation period. In general, rat milk elements (iron, copper, zinc, manganese) show a similar pattern of high initial levels, mainly during the colostrum phase, that decrease throughout lactation. The concentration of some elements increases during the last phase of lactation (iron, manganese). The iron concentration of rat milk is considerably higher than that found in human milk, and is much greater than the rat plasma iron levels. The concentrations of iron in rat milk decrease rapidly during the first part of lactation (approx. 40% drop). Thereafter, the concentration of iron continues to decrease but in a less pronounced manner. In the last days of lactation (Days 25-28), the milk iron increases. A decrease in iron content with lactation time was also found in humans, although the percentage decrease is not that high. Colostrum copper concentration are considerably higher than that of humans and decreases during the first 7 days of lactation. Copper concentration continues to decrease until Day 11, but to a lesser extent, and then remains relatively unchanged to the end of lactation. The change in copper concentration pattern is similar to that observed in humans, however copper concentration in rat is much higher. Zinc concentration is high during the colostrum period and decreases significantly during the course of lactation. Similar to copper, the change in zinc concentration pattern is similar to that observed in humans, however zinc concentration in rat is much higher. The concentration of manganese decreases significantly from Day 0 to Day 12 and remains low, but increases to nearly initial levels at the last days of lactation. Similar pattern of manganese concentration was reported in

humans. In contrast to other elements, the concentration of manganese in rat milk is not considerably higher than that of humans. Magnesium concentration was fairly stable during early and mid-lactation, but decreases during late lactation. The protein and calcium concentration increase steadily during lactation till day 24 and decreases at the end of the lactation period. The similar patterns of calcium and protein is likely due to the fact that the major protein of rat milk is casein, which is well known for its calcium binding capability. In contrast to rats, human calcium levels decline during lactation. Carbohydrate concentration increases during the first half of lactation, then decreases during the second half. In humans' milk carbohydrates level is much higher than in rats. In humans an increase in milk carbohydrate is also found in the early lactation period, but there is no decrease at later stages of lactation. While lactose is by far the major carbohydrate in human milk, rat milk may contain significant amounts of neuraminlactose. Quantitatively, the most important constituent in rat milk is fat. Similar to humans, the fat content of the rat milk did not exhibit a strong pattern during the lactation period [51].

Rabbits

Kits are weaned at the age of 4-5 weeks and are exclusively dependent on milk until lactation Day 18-19. Rabbit milk yield corresponds to kits weaning stage and reaches its peak around lactation days 17-21. It is important to remember that rabbits only nurse kits once or twice a day, strongly impacting the nutritional value of the milk. In general, rabbits' milk is concentrated with fat, protein and energy but nearly absent of lactose, and the composition does not vary significantly between most breeds. As in other mammalian species, the colostrum has higher protein content due to high immunoglobulin level. This increases the dry matter value of colostrum relative to rabbit milk. Protein content decreases along with the increase of milk yield during lactation. Apart from protein content, the composition of rabbit milk is quite stable during the 2nd and 3rd week of lactation. The changes in composition in the later stage of lactation are closely related with decrease in milk yield. Mineral element composition changes substantially after lactation peak. In general, rabbit milk is reach with calcium, sodium and potassium. Calcium concentration and to a lesser extent phosphorus increases with progressing lactation stage, while the effect on potassium and sodium is less clear and there are different data in different papers. Magnesium content increases with lactation stage while zinc, copper, iron and manganese decrease gradually in concentration as lactation progresses [52].

Discussion

Anatomy of the mammary gland

As expected, from a gross anatomy point of view, the variations between the analysed species are very high. When looking at the number and the position of glands, NHPs better resemble the human

situation, with differences only in the ducts. The number of teats is directly related to the number of the offspring [16], therefore such situation was to be expected. Pigs are on the opposite side of the spectrum as, depending on the breed and in light of the pork production requests, sows can deliver up to 18 piglets. Regarding canals/ducts, the human mammary gland is the one with the highest number out of the species taken into account, followed by dogs. A peculiar situation can be noted in rodents, were only 1 canal per teat is present. When selecting a relevant animal model for trials involving lactation, gross anatomy is not necessarily one of the key decisional factors. Indeed, having a higher number of mammary glands and teats, often allows for easier sampling procedures and higher volume specimens. From a logistics perspective, it would be indeed easy to collect samples from one teat without disturbing the animal in the feeding process if more teats are available.

Physiology of lactation

No major discrepancies were noticed regarding the physiology of lactation amongst the analysed animals. Indeed, hormonal inputs and pathways are pretty much conserved, with prolactin and oxytocin being pivotal. Nonetheless, PRL seems to play different roles in rodents when compared to the other species analysed in the present literature scanning. When looking at the most important veterinary textbooks for such topic, the bovine species is always the most representative since its role in the milk industry for human consumption is undeniable. This represents a relative limitation for experimental animals, and in particular laboratory animals, since the peculiar gastrointestinal apparatus of ruminants changes the metabolic scenario of milk production. Several knowledge gaps need to be filled regarding physiology of lactation, both at systemic and molecular levels, including in—depth characterization of hormonal mechanisms of action and environmental influences among the others.

Colostrum and milk composition

The comparison of colostrum/milk compositions between the different species can be hard to interpret in light of several technical and physiological issues. Indeed, a lot of the retrieved papers are relatively old and rely on poor sample sizes and different, often outdated, analytical tools. Moreover, high interindividual variations, mainly qualitative, within the same species are extremely common and depend on a wide variety of factors including maternal nutritional status and diet, number of newborns, or duration of lactation. Colostrum of all species contains high levels of IgG and other immune factors which increase the protein content. The level of IgG decreases within a few days post partum. As for the other components of both milk and colostrum, they vary between species and the NHPs seem to better resemble humans' mammary gland secretions, definitely more diluted when compared to the other species like rabbits, rats and mice. This finding was to be expected since, as already mentioned, the number of newborns and maternal care are amongst the key factors. Nonetheless, also (mini)pigs

show a relatively close similarity with humans' milk gross composition despite their capability to produce high litter sizes.

Practical Considerations

Choosing an animal model for a particular trial is always difficult and often implies the necessity to cope with knowledge and literature gaps. The topics hereby covered represent the starting point for a conscious decision, since anatomical and physiological similarities to humans are the basis to a high translational value experimentation. Looking at the results, NHPs, as expected, seem to represent the best model to enrol in studies regarding lactation. Nonetheless, other factors have to be considered, two of the most important being the ethical and the economical one. In the last decade, the scientific community has come to the agreement of using experimental NHPs only when strictly necessary [77]. Moreover, trials involving NHPs are expensive, long, and due to ethical considerations have low sample size that can undermine the outcome data. Finally, collecting milk samples from such species could be extremely difficult in light of the maternal behaviour. Ease of samples collection should always be a key factor when designing animal trials, as choosing a "difficult" species may lead to failure especially in "longer" trials that require un-sedated animals and/or repeated samples. Smaller animals such as rodents and rabbit are not necessarily the best choice for lactation studies. Indeed, in order to collect milk from rodents, one of the few options is the euthanasia of pups to collect gastric content right after suckling. Other methods, such as using mini-pumps to collect milk directly from the mother, often require hormonal injection to increase milk volume, a strong bias in drug lactation transfer studies, and still lead to small volume of samples. Furthermore, their milk composition resulted quite different form humans' one, and discrepancies in PRL production and functions were highlighted. In such scenario, larger animals like dogs and pigs seem to find a good fit for lactation trials. With regards to dogs, it is important to acknowledge that, despite their relevance for regulatory toxicology studies, their use for biomedical research often raises strong criticism and ethical issues in public opinion, especially in Europe. On the other hand, the enrolment of pigs in research trials seems to be more widely accepted and is considered as a valid alternative when anatomical/physiological differences are not relevant [78]. Minipigs in particular offer all the advantages of conventional pigs including genetic and metabolic similarities to humans, avoiding the main problem represented by the size. Their use in the biomedical setting is well established and recognized, and the availability of in depth physiological characterization and the related physiology-based models, vital for results interpretation, is increasing. Moreover, Göttingen Minipigs are specifically produce for biomedical purposes, with high standardized genetic background and health status.

Conclusion

In conclusion, the present analysis of the literature confirmed the complexity of the decisional process behind the choice of an animal model for *in vivo* trials. For some of the scanned species, data were either poor or completely missing, highlighting the necessity to generate more physiological background studies for species that are routinely used in laboratory settings.

Overall, the Göttingen minipig seems to represent the better choice when looking at both physiological similarities with humans and feasibility of lactation trials.

References

1. Singh B. The Common Integument. In: Dyce, Sack, and Wensing's Textbook of Veterinary Anatomy. fifth edition. Missouri: Elsevier; 2018. p. 341–58.

2. Macias H, Hinck L. Mammary gland development. Wiley Interdiscip Rev Dev Biol. 2012;1:533–57.

3. Cardiff RD, Jindal S, Treuting PM, Going JJ, Gusterson B, Thompson HJ. 23 - Mammary Gland. In: Treuting PM, Dintzis SM, Montine KS, editors. Comparative Anatomy and Histology (Second Edition). San Diego: Academic Press; 2018. p. 487–509. doi:10.1016/B978-0-12-802900-8.00023-3.

4. Russo IH, Tewari M, Russo J. Morphology and Development of the Rat Mammary Gland. In: Jones TC, Mohr U, Hunt RD, editors. Integument and Mammary Glands. Berlin, Heidelberg: Springer; 1989. p. 233–52. doi:10.1007/978-3-642-83749-4_38.

5. Cardiff RD, Allison KH. 4 - Mammary Gland. In: Treuting PM, Dintzis SM, editors. Comparative Anatomy and Histology. San Diego: Academic Press; 2012. p. 41–52. doi:10.1016/B978-0-12-381361-9.00004-4.

6. Honvo-Houéto E, Truchet S. Indirect Immunofluorescence on Frozen Sections of Mouse Mammary Gland. J Vis Exp JoVE. 2015.

7. Silver IA. The anatomy of the mammary gland of the dog and cat. J Small Anim Pract. 1966;7:689–96.

8. Sorenmo KU, Rasotto R, Zappulli V, Goldschmidt MH. Development, Anatomy, Histology, Lymphatic Drainage, Clinical Features, and Cell Differentiation Markers of Canine Mammary Gland Neoplasms. Vet Pathol. 2011;48:85–97.

9. Nickerson SC, Akers RM. Mammary Gland Anatomy. In: Fuquay JW, Fox PF, McSweeney PLH, editors. Encyclopedia of Dairy Sciences. 2nd edition. San Diego Academic Press; 2011.

10. Szendrő Z, Szendrő K, Zotte AD. Management of reproduction on small, medium and large rabbit farms: a review. Asian-Australas J Anim Sci. 2012;25:738–48.

11. Rutteman T. Mammary glands. In: Rijnberk A, van Sluijs FJ, editors. Medical History and Physical Examination in Companion Animals. second edition. Saunders; 2008. p. 132–4.

12. Davis SR. TRIENNIAL LACTATION SYMPOSIUM/BOLFA: Mammary growth during pregnancy and lactation and its relationship with milk yield. J Anim Sci. 2017;95:5675–88.

13. Pospieszny N. The anatomical structure of sow's udder – a different point of view. :4.

14. Cline JM, Wood CE. The Mammary Glands of Macaques. Toxicol Pathol. 2008;36:134s-141s.

15. Neville MC. Anatomy and physiology of lactation. Pediatr Clin North Am. 2001;48:13–34.

16. Akers RM, Denbow DM. Lactation and animal agriculture. In: Anatomy & Physiology of Domestic Animals. Balckwell Publishing; 2008. p. 475–500.

17. Djonov V, Andres AC, Ziemiecki A. Vascular remodelling during the normal and malignant life cycle of the mammary gland. Microsc Res Tech. 2001;52:182–9.

18. Gorden PJ, Timms LL. Lactation. In: Reece WO, editor. Duke's Physiology of Domestic Animals. 13th edition. Wiley Blackwell; 2015. p. 694–714.

19. Hurley WL. Review: Mammary gland development in swine: embryo to early lactation. animal. 2019;13:s11–9.

20. Cardy RH. Sexual dimorphism of the normal rat mammary gland. Vet Pathol. 1991;28:139–45.

21. Truchet S, Honvo-Houéto E. Physiology of milk secretion. Best Pract Res Clin Endocrinol Metab. 2017;31:367–84.

22. Kulski JK, Hartmann PE. Changes in human milk composition during the initiation of lactation. Aust J Exp Biol Med Sci. 1981;59:101–14.

23. Sjaastad OV, Sand O, Hove K. Reproduction. In: Physiology of Domestic Animals. 2nd edition. Oslo: Scandinavian Veterinary Press; 2010. p. 683–734.

24. Ostrom KM. A review of the hormone prolactin during lactation. Prog Food Nutr Sci. 1990;14:1–43.

25. Ben-Jonathan N, LaPensee CR, LaPensee EW. What can we learn from rodents about prolactin in humans? Endocr Rev. 2008;29:1–41.

26. Sriraman NK. The Nuts and Bolts of Breastfeeding: Anatomy and Physiology of Lactation. Curr Probl Pediatr Adolesc Health Care. 2017;47:305–10.

27. Goff JP. The Endocrine System. In: Reece WO, editor. Dukes' Physiology of Domestic Animals. 13th edition. Wiley Blackwell; 2015. p. 617–54.

28. Vieira AM, de Almeida Brasiel PG, Ferreira MS, Mateus K, Figueiredo MS, Lisboa PC, et al. Maternal soybean diet during lactation alters breast milk composition and programs the lipid profile in adult male rat offspring. Endocrine. 2018;60:272–81.

29. Görs S, Kucia M, Langhammer M, Junghans P, Metges CC. Technical note: Milk composition in mice--methodological aspects and effects of mouse strain and lactation day. J Dairy Sci. 2009;92:632–7.

30. McClellan HL, Miller SJ, Hartmann PE. Evolution of lactation: nutrition v. protection with special reference to five mammalian species. Nutr Res Rev. 2008;21:97–116.

31. Peaker M, Taylor JC. Milk secretion in the rabbit: changes during lactation and the mechanism of ion transport. J Physiol. 1975;253:527–45.

32. Chastant-Maillard S, Aggouni C, Albaret A, Fournier A, Mila H. Canine and feline colostrum. Reprod Domest Anim Zuchthyg. 2017;52 Suppl 2:148–52.

33. Oftedal OT. Lactation in the dog: milk composition and intake by puppies. J Nutr. 1984;114:803–12.

34. Kooistra HS, Okkens AC. Secretion of prolactin and growth hormone in relation to ovarian activity in the dog. Reprod Domest Anim Zuchthyg. 2001;36:115–9.

35. Mol JA, Selman PJ, Sprang EP, van Neck JW, Oosterlaken-Dijksterhuis MA. The role of progestins, insulin-like growth factor (IGF) and IGF-binding proteins in the normal and neoplastic mammary gland of the bitch: a review. J Reprod Fertil Suppl. 1997;51:339–44.

36. Farmer C, Devillers N, Rooke JA, Le Dividich J. Colostrum production in swine: from the mammary glands to the piglets. Perspect Agric Vet Sci Nutr Nat Resour. 2006;003.

37. Hurley WL. Composition of sow colostrum and milk. In: Farmer C, editor. The gestating and lactating sow. 2015. p. 193–230.

38. Morris M, Stevens SW, Adams MR. Plasma oxytocin during pregnancy and lactation in the cynomolgus monkey. Biol Reprod. 1980;23:782–787.

39. McManaman JL, Neville MC. Mammary physiology and milk secretion. Adv Drug Deliv Rev. 2003;55:629–41.

40. Wickes IG. A History of Infant Feeding. Arch Dis Child. 1953;28:151–8.

41. Sjaastad OV, Sand O, Hove K. The Endocrine System. In: Physiology of Domestic Animals. 2nd edition. Oslo: Scandinavian Veterinary Press; 2010. p. 219–58.

42. Concannon P, Tsutsui T, Shille V. Embryo development, hormonal requirements and maternal responses during canine pregnancy. J Reprod Fertil Suppl. 2001;57:169–79.

43. Hennighausen L, Robinson GW. Think globally, act locally: the making of a mouse mammary gland. Genes Dev. 1998;12:449–455.

44. Topper YJ, Freeman CS. Multiple hormone interactions in the developmental biology of the mammary gland. Physiol Rev. 1980;60:1049–106.

45. Jahn GA, Edery M, Belair L, Kelly PA, Djiane J. Prolactin receptor gene expression in rat mammary gland and liver during pregnancy and lactation. Endocrinology. 1991;128:2976–84.

46. Koiter TR, Moes H, Valkhof N, Wijkstra S. Interaction of late pregnancy and lactation in rats. Reproduction. 1999;115:341–347.

47. Rocca M, Morford LL, Blanset DL, Halpern WG, Cavagnaro J, Bowman CJ. Applying a weight of evidence approach to the evaluation of developmental toxicity of biopharmaceuticals. Regul Toxicol Pharmacol RTP. 2018;98:69–79.

48. Moffat GJ, Retter MW, Kwon G, Loomis M, Hock MB, Hall C, et al. Placental transfer of a fully human IgG2 monoclonal antibody in the cynomolgus monkey, rat, and rabbit: a comparative assessment from during organogenesis to late gestation. Birth Defects Res B Dev Reprod Toxicol. 2014;101:178–88.

49. Chastant S, Mila H. Passive immune transfer in puppies. Anim Reprod Sci. 2019;207:162–70.

50. Rooke JA, Bland IM. The acquisition of passive immunity in the new-born piglet. Livest Prod Sci. 2002;78:13–23.

51. Keen CL, Lönnerdal B, Clegg M, Hurley LS. Developmental changes in composition of rat milk: trace elements, minerals, protein, carbohydrate and fat. J Nutr. 1981;111:226–36.

52. Maertens L, Lebas F, Szendrö Z. Rabbit milk: A review of quantity, quality and non-dietary affecting factors. World Rabbit Sci. 2006;14:205–30.

53. Adkins Y, Lepine AJ, Lönnerdal B. Changes in protein and nutrient composition of milk throughout lactation in dogs. Am J Vet Res. 2001;62:1266–72.

54. Csapó J, Martin TG, Csapó-Kiss ZS, Házas Z. Protein, fats, vitamin and mineral concentrations

in porcine colostrum and milk from parturition to 60 days. Int Dairy J. 1996;6:881–902.

55. Lönnerdal B, Keen CL, Glazier CE, Anderson J. A longitudinal study of rhesus monkey (Macaca mulatta) milk composition: trace elements, minerals, protein, carbohydrate, and fat. Pediatr Res. 1984;18:911–4.

56. Mangel L, Ovental A, Batscha N, Arnon M, Yarkoni I, Dollberg S. Higher Fat Content in Breastmilk Expressed Manually: A Randomized Trial. Breastfeed Med Off J Acad Breastfeed Med. 2015;10:352–4.

57. Yamawaki N, Yamada M, Kan-no T, Kojima T, Kaneko T, Yonekubo A. Macronutrient, mineral and trace element composition of breast milk from Japanese women. J Trace Elem Med Biol Organ Soc Miner Trace Elem GMS. 2005;19:171–81.

58. Kociszewska-Najman B, Borek-Dzieciol B, Szpotanska-Sikorska M, Wilkos E, Pietrzak B, Wielgos M. The creamatocrit, fat and energy concentration in human milk produced by mothers of preterm and term infants. J Matern-Fetal Neonatal Med Off J Eur Assoc Perinat Med Fed Asia Ocean Perinat Soc Int Soc Perinat Obstet. 2012;25:1599–602.

59. Yonekubo A, Honda S, Okano M, Takahashi K, Yamamoto Y. Dietary fish oil alters rat milk composition and liver and brain fatty acid composition of fetal and neonatal rats. J Nutr. 1993;123:1703–8.

60. Auestad N, Korsak RA, Bergstrom JD, Edmond J. Milk-substitutes comparable to rat's milk; their preparation, composition and impact on development and metabolism in the artificially reared rat. Br J Nutr. 1989;61:495–518.

61. Oftedal OT, Iverson SJ. CHAPTER 10 - Comparative Analysis of Nonhuman Milks: A. Phylogenetic Variation in the Gross Composition of Milks. In: Jensen RG, editor. Handbook of Milk Composition. San Diego: Academic Press; 1995. p. 749–89. doi:10.1016/B978-012384430-9/50035-4.

62. Lönnerdal B, Keen CL, Hurley LS, Fisher GL. Developmental changes in the composition of Beagle dog milk. Am J Vet Res. 1981;42:662–6.

63. Luick JR, Parker HR, Andersen AC. Composition of beagle dog milk. Am J Physiol. 1960;199:731–2.

64. Nishikawa I, Kawanishi G, Cho F, Honjo S, Hatakeyama T. Chemical composition of cynomolgus monkey milk. Jikken Dobutsu. 1976;25:253–64.

65. Goto K, Fukuda K, Senda A, Saito T, Kimura K, Glander KE, et al. Chemical characterization of oligosaccharides in the milk of six species of New and Old World monkeys. Glycoconj J. 2010;27:703–15.

66. Hinde K, Power ML, Oftedal OT. Rhesus macaque milk: magnitude, sources, and consequences of individual variation over lactation. Am J Phys Anthropol. 2009;138:148–57.

67. Osthoff G, Hugo A, de Wit M, Nguyen TPM, Seier J. Milk composition of captive vervet monkey (Chlorocebus pygerythrus) and rhesus macaque (Macaca mulatta) with observations on gorilla (Gorilla gorilla gorilla) and white handed gibbon (Hylobates lar). Comp Biochem Physiol B Biochem Mol Biol. 2009;152:332–8.

68. Holt C, Jenness R. Interrelationships of constituents and partition of salts in milk samples from eight species. Comp Biochem Physiol A. 1984;77:275–82.

69. Young BE, Borman LL, Heinrich R, Long J, Pinney S, Westcott J, et al. Effect of Pooling Practices and Time Postpartum of Milk Donations on the Energy, Macronutrient, and Zinc Concentrations of Resultant Donor Human Milk Pools. J Pediatr. 2019;214:54–9.

70. Alves Peixoto RR, Bianchi Codo CR, Lacerda Sanches V, Guiraldelo TC, Ferreira da Silva F, Ribessi RL, et al. Trace mineral composition of human breast milk from Brazilian mothers. J Trace Elem Med Biol Organ Soc Miner Trace Elem GMS. 2019;54:199–205.

71. Keikha M, Bahreynian M, Saleki M, Kelishadi R. Macro- and Micronutrients of Human Milk Composition: Are They Related to Maternal Diet? A Comprehensive Systematic Review. Breastfeed Med Off J Acad Breastfeed Med. 2017;12:517–27.

72. Tilden CD, Oftedal OT. Milk composition reflects pattern of maternal care in prosimian primates. Am J Primatol. 1997;41:195–211.

73. Power ML, Oftedal OT, Tardif SD. Does the milk of Callitrichid monkeys differ from that of larger anthropoids? Am J Primatol. 2002;56:117–27.

74. Knight CH, Maltz E, Docherty AH. Milk yield and composition in mice: effects of litter size and lactation number. Comp Biochem Physiol A. 1986;84:127–33.

75. Riley L, Zubair M, Thomson P, Holt M, Xavier S, Wynn P, et al. Lactational performance of Quackenbush Swiss line 5 mice. J Anim Sci. 2006;84:2118–25.

76. Ragueneau S. Early development in mice. IV: Quantity and gross composition of milk in five inbred strains. Physiol Behav. 1987;40:431–5.

77. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposesText with EEA relevance. :47.

78. Hasiwa N, Bailey J, Clausing P, Daneshian M, Eileraas M, Farkas S, et al. Critical evaluation of the use of dogs in biomedical research and testing in Europe. ALTEX. 2011;28:326–40.