ASPECTS OF PLACENTOPHAGY

Original data and literature review

Dissertation zur Erlangung des akademischen Grades

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Abbreviations

ACTH	adrenocorticotropic hormone
ANP	atrial natriuretic peptide
As	arsenic
Asymp.	Asymptotic significance
BHI	brain-heart infusion
C	Celsius
Cd	cadmium
CG	chorionic gonadotropin
CGRP	calcitonin gene-related peptide
cm	centimeter
CRH	Corticotropin-releasing hormone
E1	estrone
E2	estradiol
E3	estriol
g	gram
GH	growth hormone
GnRH	gonadotropin releasing hormone
Fe	iron
FSH	follicle stimulating hormone
Hb	hemoglobin
Hg	mercury
HIV	human immunodeficiency virus
HPA	hypothalamic-pituitary-adrenal
HPE	human placental extract
hPL	human placental lactogen
IDA	iron deficiency anemia
i.m.	intra muscular
LH	luteinizing hormone
mg	milligram
µg	microgram
ng	Nano gram
Pb	lead
pCRH	placental Corticotropin-releasing hormone

pg	pictogram
PL	placental lactogen
PPD	postpartum depression
PRL	placental prolactin
r	raw
r-dhy	raw dehydrated
RT	room temperature
s	steamed
sp	species
spp	species pluralis
Se	selenium
s-dhy	steamed dehydrated
TCM	traditional Chinese medicine
TRH	thyrotropin-releasing hormone
TSH	thyroid-stimulating hormone
VIP	vasoactive intestinal peptide
YES	yeast estrogen screening
y	year

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Zusammenfassung

Einleitung:

In den letzten Jahrzehnten kann man unter Müttern der industrialisierten Länder den Trend beobachten, die eigene Plazenta als Heilmittel im Wochenbett einzunehmen. Ausgehend von den USA wurde das Verarbeiten der getrockneten Plazenta zu Kapseln für die postpartale Einnahme auch in Europa populär. Angebliche Benefits sind vermehrte Milchbildung, stabile Gemütslage, beschleunigte Rückbildung und das subjektive Gefühl von „mehr Energie" im Wochenbett.

In der traditionellen Chinesischen Medizin wird gedämpfte, getrocknete placenta hominis seit Jahrhunderten als Therapeutikum angewendet, unter anderem bei insuffizienter Milchbildung. Auch in historischen deutschen Arzneimittellehren ist getrockenete placenta hominis als wehenförderndes Heilmittel verzeichnet.

Ziel der Studie:

In dieser Studie wurde untersucht, welche Hormone und Spurenelemente in Plazentagewebe enthalten sind und wie sich deren Konzentration durch das Verarbeiten und Dehydrieren verändert. Außerdem wurde die bakterielle Kontamination von Plazenta-Präparaten analysiert.

Methoden:

Neun Plazenten, alle von spontanen, interventionslosen Geburten gesunder Frauen wurden verarbeitet: sechs wurden zur Analyse der Hormon- und Spurenelementkonzentrationen ausgewählt, acht wurden mikrobiologisch untersucht. Die Konzentrationen von CRH, hPL, Oxytocin (OT) und ACTH in rohem, dehydriertem und gedämpften und dehydriertem Plazentagewebe wurden mittels ELISA gemessen. Durch einen Hefe-Rezeptor-Bioassay wurde die Bindung der Inhaltsstoffe an den humanen Rezeptor für Östrogene bzw. Progesteron analysiert und die Östrogen- bzw. Progesteron-Äquivalente (EEQ bzw. PEQ) bestimmt.

Spurenelemente (As, Cd, Fe, Pb, Se, Hg) wurden mittels ICP-MS quantifiziert.

Isolierte Kolonien von Proben und Abstrichen der Plazentapräparate wurden durch Vitek-MS identifiziert.

Ergebnisse:

Folgende Hormonkonzentrationen (Mittelwert) wurden in roher Plazenta gemessen: CRH 177.88ng/g, hPL 17.99mg/g, Oxytocin 85.10pg/g, ACTH 2.07ng/g, Östrogen Äquivalent aktive Substanzen 46.95ng/g und Gestagen Äquivalent aktive Substanzen 2.12µg/g. Das Verarbeiten (Dampfgaren und Dehydrieren) verursachte eine signifikante Hormonreduktion.

Mikroorganismen der vaginalen Flora wurden auf Abstrichen und in den Präparat-Proben identifiziert. Die absolute Anzahl an Spezies änderte sich deutlich mit der Verarbeitung: rohe Plazenta (n=13 Spezies), roh dehydrierte Plazenta (n=5 Spezies), gedämpfte Plazenta (n=4 Spezies) und gedämpfte, dehydrierte Plazenta (n=2 Spezies).

Die Konzentrationen potentiell toxischer Spurenelemente (As, Cd, Hg, Pb) waren unterhalb der zugelassenen Höchstgehalte für Lebensmittel.

Diskussion:

Das Dehydrieren von Plazentagewebe verursacht sowohl eine signifikante Reduktion der Hormonkonzentrationen als auch eine deutliche Keimreduktion. Das Risiko für eine Intoxikation bei individueller Einnahme von Plazenta-Präparaten ist gering.

Plazentagewebe ist eine Quelle natürlicher Hormone, Spurenelemente und essentieller Aminosäuren - die Einnahme roher oder dehydrierter Plazenta könnte die postpartale Genesung, Laktation, Gemütslage und Rückbildung beeinflussen. Weitere Studien zur biologischen Verfügbarkeit der Hormone nach oraler Einnahme und deren potentiellem physiologischen Effekt sind notwendig, um die Anwendung von Plazenta-Präparaten besser beurteilen zu können.

1. Abstract

Introduction:

A growing trend among women of Western societies of consuming their own placenta after delivery, referred to as human maternal placentophagy, has developed during the last decades. Purported benefits such as enhanced lactation, stabile mood in puerperium, accelerated recovery after birth and the subjective feeling of having more energy are claimed from placentophagy supporters.

The use of placenta preparations as a treatment for insufficient lactation has been known for centuries; it is documented in traditional Chinese medicine. Desiccated placental tissue was further described in German historical pharmaceutical encyclopedias as a remedy to stimulate labor and enhance placenta expulsion.

The most popular method of ingesting placenta today is through encapsulation of dehydrated, pulverized placental tissue.

Aim of Research:

This study aims to identify the impact of placental tissue preparation on hormone and trace element concentrations. Further, possible contamination of processed placenta with microbial species has been analyzed.

Methods:

A total of nine placentas, all from spontaneous, non-interventional births by healthy women have been processed: six were studied for hormone and trace element concentrations; eight were studied for microbial contamination.

The concentrations of CRH, hPL, OT and ACTH in samples of raw, steamed dehydrated and raw dehydrated placental tissue were detected using ELISA. A yeast screening was performed in order to detect estrogenic (EEQ) and gestagenic (PEQ) active substances. Elements (As, Cd, Fe, Pb, Se, Hg) were analyzed using ICP-MS. Isolated colonies from tissue and placenta swab samples were identified using Vitek MS.

Results:

The following mean hormone concentrations were detected in raw placental tissue: CRH (177.88 ng/g), hPL (17.99 mg/g), oxytocin (85.10 pg/g), ACTH (2.07 ng/g), estrogen equivalent active substances (46.95 ng/g) and gestagen equivalent active substances (2.12 μg/g). All hormones were sensitive to processing with a significant concentration reduction through steaming and dehydration.

Microorganisms mainly from the vaginal flora were detected on placenta swab samples and samples from raw, steamed, dehydrated and steamed dehydrated tissue. Raw tissue contained the highest absolute number of species detected (n=13 species), followed by raw dehydrated tissue (n=5 species), steamed tissue (n=4 species) and steamed dehydrated tissue (n=2 species).

The concentrations of potentially toxic elements (As, Cd, Hg, Pb) were below the toxicity threshold for foodstuffs, as set by the European Union.

Discussion:

The preparation process caused a significant alteration in detected hormone concentrations. Dehydration caused a hormone loss of 85.1–97.3%. Steaming followed by dehydration caused a hormone loss of 89.2–99.6%.

The concentrations in processed placenta for the potential toxic elements arsenic, cadmium, lead and mercury were detected below the toxicity threshold for foodstuffs. Microbiological analysis of samples from raw placenta and dehydrated powder showed a decimation of microbial species through steaming and dehydrating. No organisms that pose a potential risk for ingestion as defined by the foodstuff regulations of the European Union were identified.

The number of species detected in dehydrated placental tissue was low. Retained samples showed only sporadic bacterial growth. The overall risk for microbiological spoilage of dehydrated placental tissue is low.

Conclusion:

Processed placenta is a source of different hormones that may possibly have an impact on postpartum recovery, mood disorders and lactation. Further research is necessary to evaluate the bioavailability of these hormones and possible physiologic effects. The preparation process of steaming and dehydrating provokes a significant

hormone loss, yet reduces microbial contamination. The overall risk of placentophagy is low.

Limitations:

The findings of this research need to be confirmed through a repeated study with a larger sample size. Randomized, double-blind, placebo-controlled studies are necessary to determine possible physiologic influences of placentophagy to the human hormone system and to evaluate the potential use of individual placenta remedies as a postpartum treatment.

2. Introduction

2.1 Placenta Structure

Morphologically the human term placenta is a single disc with a diameter of 15–20 cm and a thickness of 2–3 cm. Amnion and chorion, the fetal membranes that surround the fetus in the amniotic cavity, extend from the margins of the chorionic disc. The placental parenchyma is divided into irregular cotyledons by protrusions of the basal plate (Benirschke and Kaufmann 1995). The embryo/fetus is connected to the placenta through the umbilical chord.

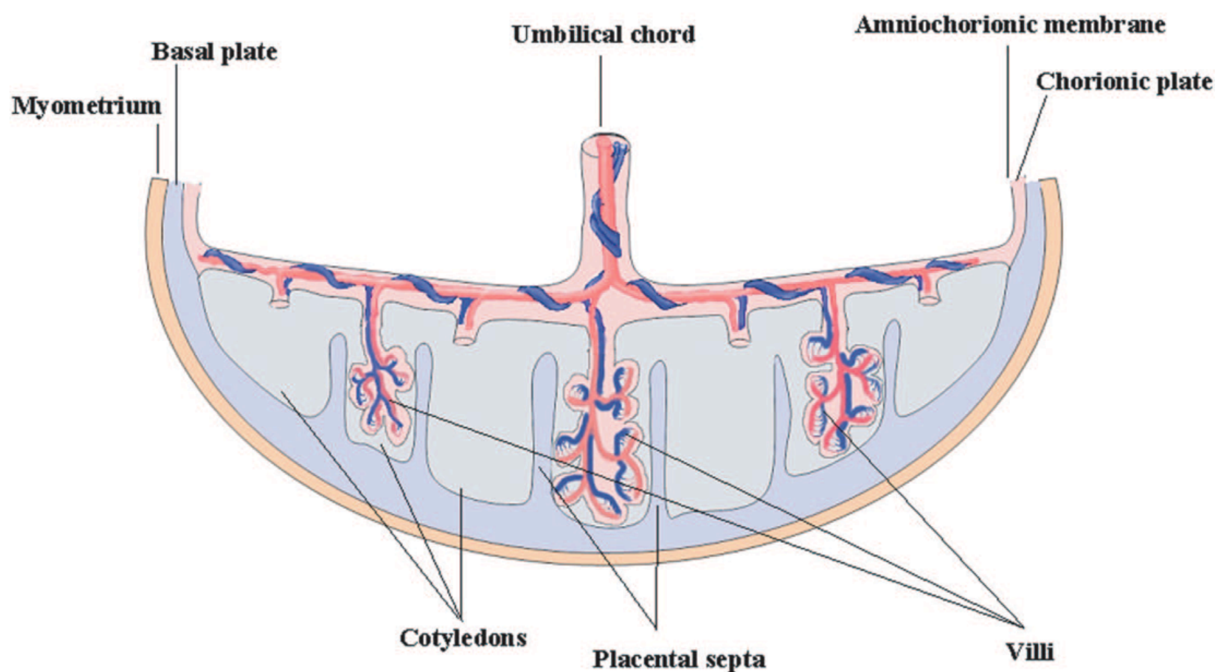

Figure 1: Schematic section of the human term placenta.
Placental septa are produced by protrusions of the basal plate from the maternal side of the placenta; the parenchyma is divided into irregular cotyledons. Each cotyledon contains several villi, which originate from the chorionic plate. Fetal blood vessels are located within the branches of the villi. Reprinted with permission (Parolini et al. 2008).

The placenta separates the maternal and the embryonic/fetal blood from each other. Maternal cells and embryonic/fetal tissue are always separate. This is necessary to avoid maternal immune defense towards the fetus (Kliman 2000).

During the very early stages of pregnancy the blastocyst, a rotund structure, differentiates into two separate cell types: An inner cell mass that becomes the fetus (embryoblast), and an outer cell mass with undifferentiated trophoblast stem cells that will develop into external membranes and the placenta (Geneser 1986). The cytotrophoblast can be described as the stem cell of the placenta. Four days after fertilization, it emerges as the outer layer of the blastocyst. The cytotrophoblast consists of undifferentiated mononuclear cells. Different forms of trophoblasts evolve from the cytotrophoblast:

The *villous syncytiotrophoblast* is the site of endocrine activity and hormone production. Another type, the *junctional trophoblast*, ensures attachment of chorionic villi onto the maternal decidua by producing trophouteronectin (Feinberg et al. 1991). Cells of the *invasive intermediate trophoblast* leave the placenta to migrate into the decidua, the myometrium, and finally into the spiral arteries of the maternal uterus (Kliman 2000). The invasion of spiral arteries leads to increased blood flow to the placental bed.

The decidualization of the uterine epithelium makes it receptive for the activated blastocyst to implant. The lining of the maternal uterine lumen, excluding the endometrium beneath the implanted blastocyst, is called the decidua parietalis. The cells of the endometrial stratum functionale proliferate and grow into large, polyhedral decidual cells, building the decidua basalis (Geneser 1986). The decidua as a tight cellular matrix functioning as a barrier to prevent the penetration of invasive trophoblast cells. Implantation and placentation is a balance between trophoblast and endometrium interaction (Kliman 2000). After the implantation of the blastocyst into the decidua basalis, the endometrium closes superficially through a compact layer called the decidua capsularis.

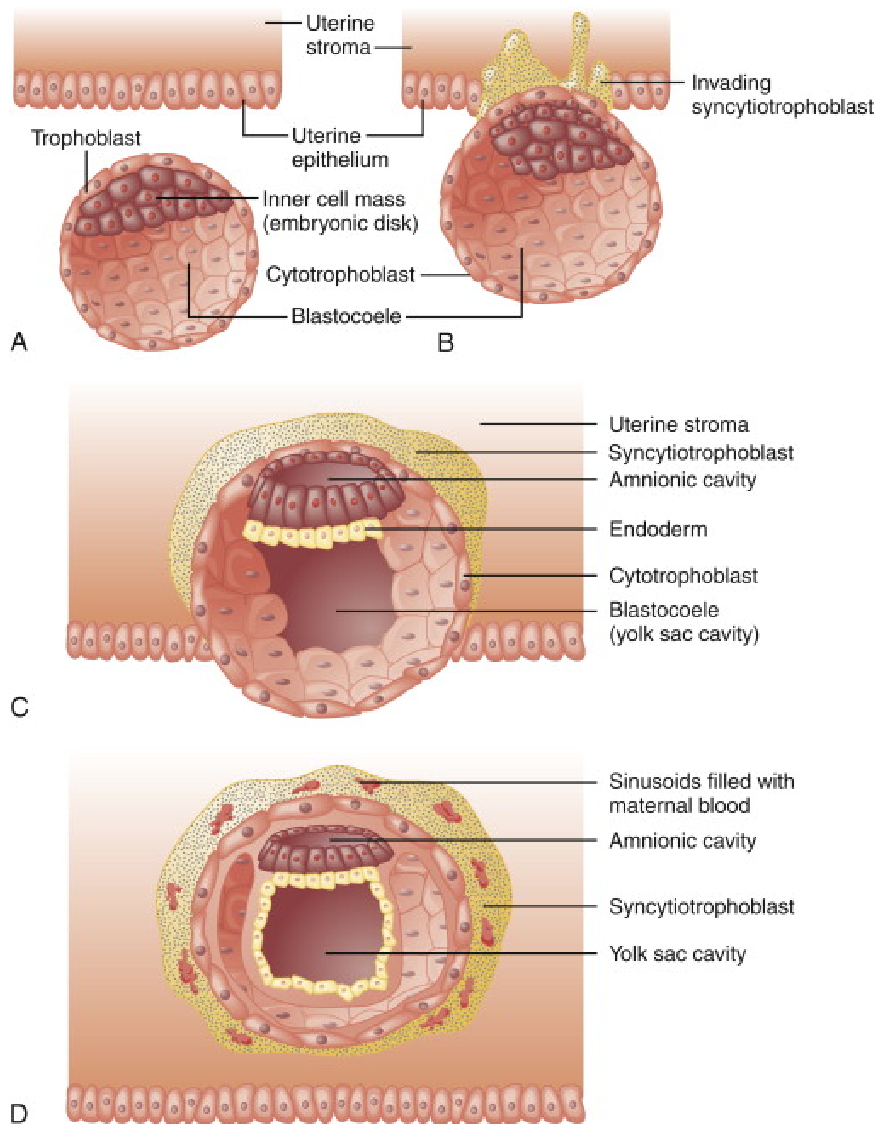

Figure 2: The implantation of the human embryo.
A. Floating blastocyst. B. Attachment to the uterine epithelium and initial invasion of the syncytiotrophoblast cells. C. The blastocyst penetrates deeper into the uterine stroma and develops an amniotic cavity. D. The fully implanted embryo invades the maternal vasculature and the uterine epithelium grows over the implantation site and undergoes decidualization. Reprinted with permission from Jones RE. 1997 Human Reproductive Biology by Academic Press, San Diego, p 189.

The human placenta is a hemochorial organ. This means that chorionic epithelial layers form villi that lie in the maternal blood for extensive nutrition and gas exchange.

The trophoblast invades the endothelial layer of the maternal endometrial blood vessels, forming intertrophoblastic, maternal blood-filled sinuses. Thus the placental villi float freely in maternal blood (Enders and Welsh 1993).

Three weeks after fertilization the first sight of fetal circulation is evident. Capillary loops within chorionic villi penetrate the intervillous space. The embryonic blood is carried to the villi by the umbilical arteries, returning after the exchange of metabolic products to the embryo by the umbilical vein (Geneser 1986, Kliman, 2000 #100).

2.2 Stem Cells

The human placenta is of great medical interest because it consists of progenitor/stem cells (Kyurkchiev et al. 2012). Obtaining embryo-derived, pluripotent cells is the point of origin for regenerative medicine (Mason and Dunnill 2008).

The term “stem cell” has been used in medical literature to describe a number of cells isolated from placenta that demonstrate phenotypic plasticity. Phenotypic plasticity is a common characteristic of the reaction norm of a genotype. Plasticity describes the possibility of appearing as a novel, environmentally induced phenotype (Pigliucci et al. 2006). The following cell populations of the placenta possess this ability: *human amniotic epithelial cells* (hAEC), *human amniotic mesenchymal stromal cells* (hAMSC), *human chorionic mesenchymal stromal cells* (hCMSC), and *human chorionic trophoblastic cells* (hCTC) (Parolini et al. 2008).

Other features of “stem cells” are self-renewal and hierarchy. These cells are at the apex of the cell system because all cell types can develop from them. Used in regenerative medicine these cells could repair or replace damaged or malfunctioning cells and tissues.

2.3 The difference between human and mouse placenta

Much research has been done on placenta using mouse models. Studies have identified similar cell types in human and rodent trophoblasts (Cross 2006). But the difference in morphology and histology of both human and murine placentas should not be forgotten.

All rodents retain a yolk sac placenta, which stays until term and becomes active early in pregnancy, preceding the chorioallantoic placenta. The yolk sac placenta is mandatory for a successful pregnancy in rodents; it is part of the fetal membranes.

Humans do not keep a yolk sac placenta: only in the very early stages of pregnancy is an umbilical vesicle formed for embryonic blood supply, although it never forms a placenta (Carter 2012).

Decidualization, the transformation of the endometrium that facilitates implantation, happens at different points of gestation in these two species. In humans, decidualization takes place before implantation (Kliman 2000); in mice, implantation is the trigger for decidualization (Enders and Welsh 1993).

The structure of the human placenta becomes apparent in early pregnancy. By day 21 of 270 the chorionic villi are established (Benirschke and Kaufmann 1995). The chorioallantoic placenta in mice is only complete halfway through gestation; the chorioallantoic pattern is seen by day 11.5 of 19–20 days of gestation (Malassine et al. 2003). A chorioallantoic placenta is supplied with blood by the allantoic vessels. It is the definite type in human and rodent placentas (Carter 2012).

The endocrine function of mice and human placenta differs widely. For maintenance of pregnancy, human chorionic gonadotropin (hCG) is indispensable in humans.

It exists in five isoforms, with hCG and hyperglycosylated hCG playing crucial roles in human pregnancy (Cole 2012). A prominent role of hCG is the stimulation of the corpus luteum to synthesize progesterone in the first 8 weeks of gestation. After that time the syncytiotrophoblast of the placenta produces sufficient progesterone for maintaining pregnancy (Srisuparp et al. 2001). In mice, no hCG exists and the corpus luteum is required throughout pregnancy as the main site of progesterone synthesis (Strauss et al. 1996).

The syncytiotrophoblast is where maternal and fetal blood is exchanged, and has a high level of endocrine activity, in the human placenta. The comparable structure for maternal-fetal exchanges in mice – the trophoblast layers of the labyrinth – is without endocrine function (Malassine et al. 2003).

However, in contrast to humans, mice possess a gene cluster of 23 prolactin/placental lactogen-related genes while humans only have one gene for prolactin. In humans, these hormones are produced by the syncytiotrophoblast, in mice, it is produced by trophoblast giant cells, the spongiotrophoblast: glycogen cells that represent trophoblast layers. These are absent from humans (Simmons et al. 2008).

Comparing these differences between placentae of humans and mice shows that the results from placentophagy studies with murine species should not be transferred to humans without caution (Schmidt et al. 2015).

2.4 Placenta as an endocrine organ

During the period of gestation the placenta develops as a self-managed, unique organ. It is essential for maintaining pregnancy and enables fetal-maternal exchange. The placenta requires high levels of oxygen for the production of steroid, peptide and protein hormones. These regulate the adaptation of the maternal organism to pregnancy, support fetal growth, maintain the wellbeing of the embryo and prepare the maternal body for parturition. These hormones also play an important role in the local maternal-fetal immunotolerance.

Human pregnancy is an unusual hyperestrogenic and hypercortisolic state that increases with the duration of gestation and suddenly comes to an end after the delivery of the fetus and placenta. The hormonal changes during pregnancy are shown in Figure 3.

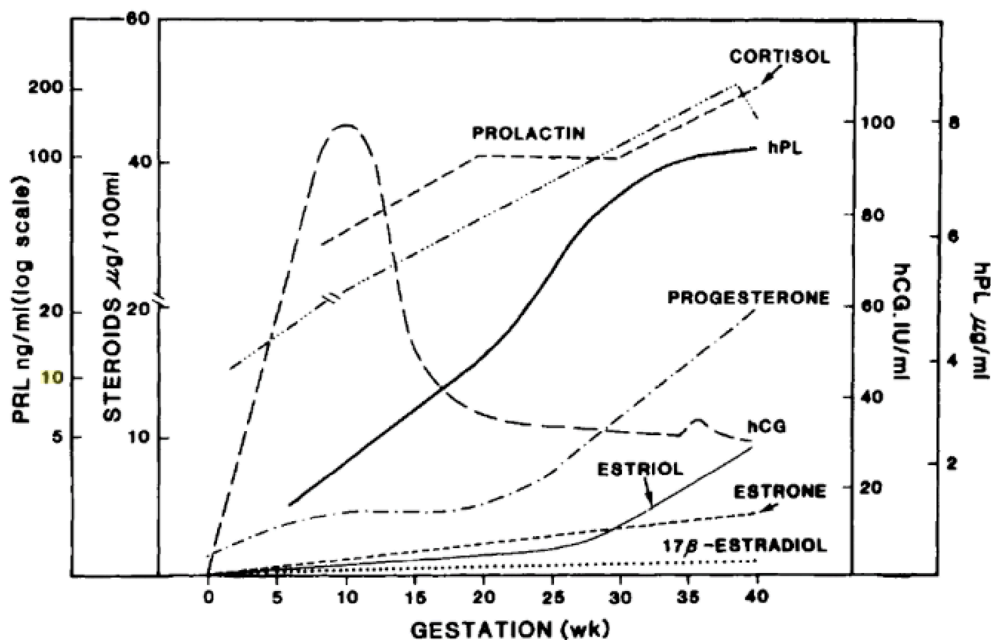

Figure 3: Temporal changes during human pregnancy in the serum concentration of several hormones. Reprinted with permission from "Hormones" by Anthony W. Norman and Gerald Litwack, Academic Press 1987.

Table 1 gives an overview of hormones produced by the placenta. Some of them are discussed in detail below.

Table 1: Placental hormones.

Table modified from (Reis und Petraglia 2001).

Neuropeptides	Pituarity-like Hormones	Steroid Hormones	Monoamines and Adrenal-like Peptides
CRH	ACTH	Progesterone	Epinephrine
TRH	TSH	Estradiol	Norepinephrine
GnRH	GH	Estrone	Dopamine
Melatonin	PL	Estriol	Serotonin
Cholecystokinin	CG		Adrenomedullin
Met-enkephalin	LH	2-Methoxyestradiol	
Dynorphin	FSH	Allopregnanolone	
Neurotensin	B-Endorphin	Pregnenolone	
VIP	Prolactin	5α-Dihydroprogesterone	
Galanin	Oxytocin		
Somatostatin	Leptin		
CGRP	Activin		
Neuropeptide Y	Follistatin		
Substance P	Inhibin		
Enothelin			
ANP			
Renin			
Angiotensin			
Urocortin			

2.4.1 Hormones

Progesterone

Progesterone is a steroid hormone. It is produced by the corpus luteum during the menstrual circle. It is the main pro-gestational hormone in pregnancy.

From the sixth week of pregnancy, progesterone is exponentially synthesized throughout subsequent gestation. The plasma concentration is increased 10-fold by the third trimester (Tulchinsky et al. 1972). The syncytiotrophoblast produces large amounts of progesterone from maternal cholesterol. A placenta produces daily about 300 mg of progesterone at term (Evain-Brion und Malassine 2003).

The effects of this hormone are various. Progesterone maintains and protects pregnancy by suppressing myometrial activity to prevent uterus contractions as well

as inducing the formation of the mucus plug to close the cervix. In addition, it has an influence on mammillary gland's preparation for lactation (Yen et al. 2014). It further has anesthetic and anticonvulsive properties through its modulation of the gamma amino butyric acid (GABA) receptor (Paul und Purdy 1992). Its role in development and treatment of postpartal depression (PPD) is not yet understood, and studies examining changes in progesterone levels in the first postpartum week report conflicting results (Abou-Saleh et al. 1998).

Estrogens

Estrone (E1), estradiol (E2) and estriol (E3) are steroid hormones. After week 7 of gestation more than 50% of the estrogens found in the maternal serum are of placental origin. The biosynthesis of placental estrogen differs from the synthesis of estrogens in other tissues of the human organism. The placenta is not able to convert C_{21} steroids to C_{19} steroids, as it lacks the enzyme 17α-hydroxylase. Trophoblasts use dehydroisoandrosterone sulphate (DHEAs) from the fetal adrenal glands for the biosynthesis of estradiol-17β (Kaufmann 1993). The cooperation between fetal adrenal glands and maternal placenta in steroid synthesis forms the concept of the feto-placental unit (Redman 1993). The fetus plays an important role for maternal estriol synthesis, as it is the source of 90% of the precursors of placental estriol.

Estrogen has multiple effects. It prepares the mammillary glands for lactation; it enhances myometrial and uterine growth, uterine and placental blood flow and prostaglandin production by the endometrium. In addition, it regulates LDL uptake for placental steroid synthesis.

Chorionic Gonadotropin - hCG

Human chorionic Gonadotropin (hCG) is a peptide hormone and belongs to the family of complex proteins known as glycoprotein hormones. It has two intertwined subunits, the common α subunit and distinct β subunit.

HCG indicates pregnancy when detected in maternal blood or urine, usually shortly after conception, that is, about 8-10 days after ovulation. It is one of the earliest secreted products of the conceptus. HCG is secreted initially from the cytotrophoblast and later from the syncytiotrophoblast. After implantation, the plasma concentration rises rapidly with a peak during the 8th to 10th week of pregnancy. It tends to plateau at a lower level for the remainder of the pregnancy (Yen et al. 2014).

In vitro studies have shown that the hCG production by the cytotrophoblast differs depending on placental age (Kato und Braunstein 1990).
HCG acts biologically in the same way as luteinizing hormone (LH) and is important for pregnancy maintenance. It inhibits the corpus luteum from involution and ensures progesterone secretion by the ovarian granulosa cells.

Human placental lactogen – hPL = Chorionic somatomammotropin

Human placental lactogen is a polypeptide hormone consisting of 191 amino acids, with a structure similar to prolactin and growth hormone (GH). It has a potent lactogenic and growth hormone-like effect (Reis et al. 2001).
Its production increases throughout pregnancy, showing a correlation with gain of placental mass, reaching an extremely high secretion rate of about 1–3g per day towards term (Evain-Brion and Malassine 2003). It has a very short half-life in maternal plasma and is secreted continuously from the placenta during late human pregnancy (Eriksson et al. 1989).
Two variations of growth hormone exist in the maternal endocrine system: GH-N, the pituitary growth hormone, and GH-V, the placental growth hormone that is produced in the syncytiotrophoblast. Both growth hormones are strong insulin antagonists that break down lipids and stimulate hepatic gluconeogenesis. The placental variant increases the nutrients available to the fetus and placenta, as well as protecting them from hypoglycemia. It is not noted in fetal tissue. GH-V is released into the maternal circulation from the second trimester onwards, gradually replacing pituitary GH-N. After delivery, placental GH-V immediately disappears from the maternal circulation, and pituitary GH-N is once again excreted (Newbern and Freemark 2011).

Placental CRH

Hypothalamic CRH is an essential neurohormone for stress adaption. It mediates the Hypothalamic-Pituitary-Adrenal-Axis (HPA-Axis) and the behavioral and autonomic response to stress (Aguilera and Liu 2012). CRH stimulates the pulsatile liberation of adenohypophysal ACTH. This induces adrenal cortisol release and synthesis.
The placental counterpart is placental CRH (pCRH). PCRH is a 41-amino acid peptide hormone with relevant endocrine and paracrine effects. It is secreted mainly from the syncytiotrophoblast.

PCRH is similar to hypothalamic CRH in structure, immunoreactivity and bioactivity, and it has an effect on the function of the HPA-Axis. It is produced exponentially throughout pregnancy; with vaginal delivery being the moment of highest reached serum levels. PCRH stimulates prostaglandin release and the release of immunoreactive oxytocin from placental cells. It also plays a role in feto-placental perfusion and has an effect on myometrial contraction. This hormone also has a direct effect on the liberation of DHEAs from adrenal cells, which is needed for placental estrogen synthesis (Reis et al. 1999).

A positive feedback mechanism forms the basis of the CRH-synthesis – a rise in fetal adrenal cortisol and maternal glucocorticoids stimulates placental CRH production (Schleussner 2002). Towards the end of gestation an increasing cortisol production of fetal adrenals stimulates placental CRH secretion. CRH induces ACTH secretion. This ascending hormonal loop can be seen as the placental response to delivery stress (Yim et al. 2009).

Chorionic adrenocorticotropin – cACTH

Chorionic or placental ACTH is a peptide hormone with the same structure and immunogenic and biologic activity as pituitary ACTH. It is synthesized at the cytotrophoblast during the first trimester, after which time the syncytiotrophoblast is the site of production. Its effect could be the stimulation of steroid synthesis in the placenta (Barnea et al. 1986).

Placental Thyrotropin- releasing hormone (TRH)

TRH secreted by the hypothalamus stimulates the release of thyroid-stimulating hormone (TSH) and prolactin. The syncytiotrophoblast produces placental TRH from the beginning of pregnancy until term. It is mainly released into fetal circulation and may regulate fetal thyroid function (Reis et al. 2001).

Chorionic thyrotropin – hCT

HCT is a TSH-like peptide with a very low thyrotropic activity. Its levels increase throughout pregnancy (Reis et al. 2001).

Placental GnRH

Placental GnRH (pGnRH) is identical to hypothalamic GnRH. It can be measured from the first trimester until term. PGnRH is believed to regulate paracrine hCG release (Iwashita et al. 1993).

Placental Growth hormone-releasing hormone (GHRH)

Hypothalamic GHRH is a peptide hormone and stimulates the release of GH from the adenohypophysis. The placental GHRH is identical to hypothalamic GHRH; it regulates fetal and placental growth during pregnancy.

Placental Prolactin

Placental prolactin (pPRL) is a peptide hormone that not only exists in the fetal pituitary but can also be found in cells of the decidua, the amnion and the chorion. The possible site of biosynthesis is in decidualized cells. The role of pPRL in pregnancy is not yet known (Wu et al. 1991).

Oxytocin

Oxytocin (OT) is a peptide hormone. If released from the posterior pituitary gland it initiates and sustains milk ejection in response to suckling during lactation
(Wakerly JB 1988). In addition, OT is a potent and specific hormone to stimulate labor, triggering myometrial contractions. OT is produced by several organs including the placenta (Kiss and Mikkelsen 2005) and its gene expression can be found in in the decidua, the amnion and the chorion. A three- to four-fold increase in the rate of synthesis of OT mRNA in human chorio-decidual tissue around the time of parturition has been demonstrated, illustrating its importance in labor (Chibbar et al. 1993).

Besides the myometrial function it has various psychosocial effects. It is the key mediator of complex emotional and social behaviors, including attachment, social recognition, and aggression (Kirsch et al. 2005).

Leptin

Leptin is a peptide hormone synthesized and secreted by adipose tissue and the maternal placenta. Placental leptin is increased significantly between the first and second trimester of pregnancy. Leptin acts on the hypothalamus and regulates food intake and energy balance. It has been suggested that several gestational hormones induce its synthesis (Sivan et al. 1998).

PSG

Pregnancy-specific glycoproteins are composed of an immunoglobulin variable-like domain and a variable number of constant-like domains. They may act to modulate

maternal immune responses and induce monocytes to secrete several anti-inflammatory cytokines.
PSG can reach concentrations of 200 – 400 µg/ml at term and are the most present placental proteins in maternal blood (Carter 2012).

Beta-Endorphins

Pregnancy is characterized by several changes in the opioid peptides. Beta-endorphin plasma levels increase progressively during gestation, reaching the highest concentration at term with a maximal value at delivery (Genazzani et al. 1981). Beta-endorphin is synthesized in human placental trophoblastic cells (Liotta und Krieger 1980). Interestingly, beta-endorphin levels were lower in cases of elective C-section compared to spontaneous births, with the presence or absence of labor as the possible critical factor (Facchinetti et al. 1990).

2.4.2 Further components of the placenta

Placental tissue at term contains a high concentration of hormones and nutrients.
De Moraes et al. studied the elemental composition of the human placenta. The following median concentrations of essential elements were found in maternal parts of the placenta in adults: calcium 700.8 ng/g, iron 85.3 ng/g, copper 0.8 ng/g
and zinc 12.7 ng/g (de Moraes et al. 2011).
The concentrations of the following trace elements have been determined in dried or wet placental tissue. Selenium, an essential trace element, has a concentration of
0.56 – 1.06 µg (Alonso et al. 2005). Cadmium, a toxic trace element, was detected at 31 ng/g (Schramel et al. 1988). It accumulates in the placenta and was found to be elevated in smoking mothers (Bush et al. 2000). Nickel had an average concentration of 36 ng/g, aluminum had an average concentration of 250 ng/g and arsenic had an average concentration of 6 ng/g. The average concentration of lead was found to be 34 ng/g (Iyengar and Rapp 2001). Chronic exposure to lead produced a modest increase in lead concentration in the placental body (Baghurst et al. 1991). The concentration of mercury was, in the majority of placentas examined by Schramel et al., below the detection limit (Schramel et al. 1988).

2.5 Placentophagy

Based on existing definitions from animal studies and ethnographic research of humans, the term placentophagia is relating to the consumption of afterbirth by animals. The behavior of ingesting placental tissue by humans is defined as human placentophagy (Marraccini und Gorman 2015).

2.5.1 Placentophagia in mammals

The greek term "Placentophagia" can be translated as "ingestion of the afterbirth". It is the behavior of eating the afterbirth, which consists of the placenta, the umbilical cord, the amniotic and chorionic membranes, and the amniotic fluids (Kristal 1980). Almost every eutherian mammalian species engages in this behavior after parturition. Humans, camelids and marine mammals are the few exceptions (Young et al. 2012). Nevertheless, it occurs in all non-human primate species (Stewart 1977). Many hypotheses have been put forward to explain placentophagy, ranging from cleaning the nest site in order to avoid attack by predators, to carnivorousness after parturition, to specific or general hunger.

Kristal (1980) disproved these assumptions, defining placentophagy as an ingestive behavior that is found in vastly different mammalian groups and consequently remains a mystery. Kristal states that it is not evident if there is a single, special advantage of placentophagy, or if the summary of multiple benefits leads to placenta ingestion.

The lactagogic effect of placentophagia in collared peccary *(Pecari tajacu)* is described by Schmidt (Schmidt 1976). Lactogenesis is induced through ingestion of the afterbirth not by the mother but by the older siblings of the newborn. These subadult females start nursing the newborn, allowing the mother to recover from delivery.

2.5.2 Placentophagy in humans

Placentophagy is remarkably uncommon in humans compared to other mammals.

An ethnographic cross-cultural study identified only 1 of 179 societies that mentioned the practice of maternal placentophagy (Young und Benyshek 2010).

There are many methods used to dispose of this organ, with burial being the most common. In 67 of the 179 examined cultures, placenta rituals are connected to superstitious beliefs (Young und Benyshek 2010).

Placentophagy in humans was described by Young as an ancestral mammalian behavior that was subsequently lost in the evolution of the homo sapiens. A possible reason why placentophagy is now absent in humans could be the change in lifestyle that came about with the directed use of fire. As stated in the "fire hypothesis" (Young et al. 2012) the accumulation of toxic trace metals in the placenta after exposure to wood smoke could be responsible for negative effects experienced after ingesting the afterbirth.

Menges investigated possible motivation for human placentophagy in evolutionary biology. She stated that there is no phylogenetic background for that behavior and no reasonable explanation in evolutionary biology (Menges 2007). Nevertheless, anecdotal reports about human placentophagy in diverse ethnos do exist (Ober 1979, Enning 2003).

Since 1973 placentophagy has been mentioned in scientific discussions. For example, Ober reported that a mother living in a commune ate her steamed placenta after giving birth naturally (Ober 1979).

In recent years there has been a growing interest in placentophagy among new mothers in industrial nations (Selander et al. 2013). Discussions about natural, holistic and spiritual childbirth, including placentophagy and its possible benefits, are increasing on the internet and in other media (BBC 2006, USA today 2007, Blumenfeld 2013). A debate about different ways of preparing placenta and recipes for placenta meals is ongoing.

2.6 Different placenta preparations

Different ways of preparing the placenta have been described in popular and medical literature. One method explained by Enning is the dehydration of the placenta in the oven until it is completely mummified. To avoid any substance changes in the placenta, the heat supply should be as low as possible. A bowl with water and vinegar should be placed below the placenta for mild sterilization (Enning 2003).

The use of acetic acid has a wide, excellent bactericidal effect, even at low concentrations (Ryssel et al. 2009).

Subsequently the black, hard placenta is crushed into pieces and ground in a mortar This way of preparing the placenta is very basic, uncomplicated and does not require much equipment (Enning 2003).

There is archaeological evidence that drying strips of meat was practiced in early civilizations. Desiccation, which means reducing the water activity of a product to a low level, was developed by the ancient Egyptians for the mummification of the deceased (Hugo 1991).

Another method is ingesting raw placenta shortly after delivery. A piece of placenta is washed in salt water, cut into thin slices and stored in the freezer. One slice of placenta is eaten per day (Enning 2003).

Several organizations have been set up in the United States that provide placenta-encapsulation services. Table 2 shows the first 20 results of a Google online search using the key words "placenta encapsulation service". These organizations offer to collect the woman's placenta immediately after childbirth and prepare placenta capsules from it.

Two different methods are used for encapsulation: "raw" and "Traditional Chinese Medicine" (TCM) encapsulation. For the production of "raw" capsules, the placenta is sliced and dried by dehydration. The dehydrated pieces are ground and put into empty vegetable capsules. For the production of "TCM" capsules, the placenta is steamed prior to dehydration.

In addition to placenta capsules, there are many other products offered by these organizations, such as placenta tincture, placenta essence, placenta salve, placenta cream, placenta tea, placenta smoothies, placenta truffles and placenta meal preparation.

Table 2: First 20 results for "placenta encapsulation" web search (21.03.2015).

Organization	Website	Location
Tree of life	www.portlandplacentaservices.com	Portland, U.S.
Afterbirth Anywhere	www.afterbirthanywhere.com	U.S.
Placenta Benefits	www.placentabenefits.info/services.asp	North Las Vegas Nevada, U.S.
Individual Placenta Encapsulation Network	www.placentanetwork.com	U.K.
Placenta Mom	www.placentamom.com	Sacramento, U.S.
Placenta Service	www.placentaservice.com	Hudson Valley, New York, U.S:
The Association of placenta preparation arts	www.placentaassociation.com	U.S.
Lucina Birth Service	www.lucina.ca/placenta.html	Toronto, Canada
Massachusetts placenta encapsulation	www.massachusettsplacentaencapsulation.com	Massachusetts, U.S.
Tree of life	www.treeoflifebirthandbeyonde.com.au	Sidney, Australia
Placenta Service	www.placentaservices.com.au	Sidney, Australia
Afterbirth Service	www.afterbirthservice.com	Michigan, U.S.
Brooklyn Placenta Service	www.brooklynplacentaservices.com	Brooklyn, New York City, U.S.
Hampton Roads Placenta Encapsulation	www.hrplacenta.com	North Carolina, U.S.
Placenta Encapsulation Service	www.bywaterbirth.com/encap.html	Minnesota, U.S.
Richmond Placenta Encapsulation	www.richmondplacentaencapsulation.com	Virginia, U.S.
The Nurturing Root	www.thenurturingroot.com	Maryland, U.S.
Wise Birth Choices	www.wisebirthchoices.com/placenta.html	New Jersey, U.S.
Minnesota Placenta Service	www.flutterbybirth.com/placenta-services.html	Minneapolis, Minnesota, U.S.

2.7 Traditional use of placenta remedies

The use of dried human placenta, known as “zi he che”, has a long tradition in Traditional Chinese Medicine (TCM). As a TCM therapeutic it tonifies the liver and kidney for the treatment of infertility, impotence and spermatorrhea. Dried human placenta augments the "qi" (body energy) and nourishes the blood as a cure for emaciation and insufficient lactation (Bensky et al. 1986).

The use of desiccated placental tissue for complicated labor was further described in the *Pharmacopoeia Wirtenbergica*, a German historical pharmaceutical encyclopedia as following: Secundinarium humanarum praeparatio – usus est in pulveribus, ad partum difficilem (Gesner 1741).
Additionally, in 1743 the use of powder from dehydrated human placenta to stimulate labor and enhance placenta expulsion was described in a historic medical encyclopedia (Schmidt 1743).

2.8 Microbiology of the female genital system

2.8.1 The microbial composition of the vagina

The vaginal ecosystem is complex and dynamic, with changes throughout a women´s lifespan from birth, to puberty and menopause. Compared to non-pregnant women, the vaginal microbiota is more stable during pregnancy (Romero et al. 2014). Aagard and colleagues have shown that the microbial community is less diverse and rich during pregnancy (Aagaard et al. 2012).
The healthy vaginal flora in pregnant women is *Lactobacillus* predominant;
L. vaginalis, L. jensenii, L. crispatus and *L. gasseri* have a relative high abundance in pregnant women (Romero et al. 2014, Hillier et al. 1993). The predominance of Lactobacillales is followed by Clostridiales, Bacteriodales and Actinomycetales (Aagaard et al. 2012).
Facultative Lactobacilli make up 50–90% of the aerobic vaginal microflora (Redondo-Lopez et al. 1990). The acidic environment in the vagina is maintained by *Lactobacillus* spp., but lactic acid can similarly be produced by other microorganisms in the vagina such as *Atopobium*, *Megasphaera* and *Leptotrichia*

(Larsen und Monif 2001). Through the production of lactic acid a low vaginal pH (<4.5) is maintained, functioning as a barrier against infections by genital pathogens. The following bacterial species can be found in varying population density in the normal vaginal flora of non-pregnant women (Neumann et al. 2014):

- Lactobacilli
- *Bacteroides* sp.
- Corynebacteria
- *Porphyromonas* sp.
- *Prevotella* sp.
- Enterobacteriaceae
- Enterococci
- *Fusobacterium* sp.
- *Leptotrichia* sp.
- *Megasphaera* sp.
- *Gardnerella vaginalis*
- *Mobiluncus curtisii*, *M. mulieris*
- *Mycoplasma hominis*
- *Ureaplasma urealyticum*
- Peptostreptococci
- Propionibacteria
- Pseudomonadaceae
- Streptococci (not *Streptococcus*-A)
- Staphylococci

Microorganisms recovered from pregnant women with bacterial vaginosis included *Gardnerella vaginalis, Bacteroides* spp*, Peptostreptococcus* spp (Hillier et al. 1993). Other pathogenic species are *Neisseria gonorrhoeae*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenza*, *Listeria monocytogenes* and *Trichomonas vaginalis*. Furthermore, the vaginal flora can be disturbed by aerobic microorganisms such as *E. coli*, Group B Streptococci, Enterococci and others (Hillier et al. 1993). Carriage of these microorganisms does not necessarily constitute disease, when disease is defined in terms of symptoms. But they bring the potential for disease to the vaginal area (Larsen und Monif 2001). Similarly, the yeast *C. albicans* is present in the vagina of approximately 10–20% of women of reproductive age. The carriage is typically asymptomatic (Witkin et al. 2007)

2.8.2 The placental microbiome

Not only the vagina but also the placenta and endometrium harbor physiologic communities of microorganisms. The upper genital tract and endometrium is not sterile and is predominantly populated by *Lactobacillus*, followed by bacterial communities of *Gardnerella, Prevotella, Atopobium* and *Sneathia* (Franasiak und Scott 2017).
The microbiome of the placenta was characterized by Aagard et al. using metagenomic analysis. The placental microbial community represents a unique niche of organisms with several species of the oral microbiome detected including *Prevotella tannerae* and nonpathogenic *Neisseria* species. The placental microbiome is composed of commensal microbiota from Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes and Fusobacteria phlya (Aagaard et al. 2014).

2.9 Placentophagy: Current state of research

The effects of placentophagy have been examined intensively on rats.
These investigations have shown that animals which consume their placenta display an increase in maternal pup-directed contact, enhancement of opioid-mediated antinociception and a suppression of postpartum pseudo-pregnancy.
An active substance that potentiates pregnancy-mediated analgesia was found in the placenta and the amniotic fluid. This substance has been termed Placental Opioid-Enhancing Factor, also known as POEF. It is a peptide that works via gastric vagal receptors on the central nervous opioid system before it can be denaturized in the acidophil gastric environment (DiPirro and Kristal 2004), Kristal et al. 2012).
Hormonal changes after placentophagy in rodents have also been investigated.
Rats that were allowed to eat their placenta after parturition had elevated serum prolactin concentrations on day 1, and depressed concentrations of serum progesterone on day 6 and 8 post partum, when compared to those hormone levels of rats prevented from eating their placenta.
This may indicate that rat placenta contains orally active substances that modify blood levels of pituitary and ovarian hormones (Blank and Friesen, 1980).

There are outdated studies on human maternal placentophagy by Hammett and Soykova-Pachnerova et al. However, they do not meet the criteria for standardized double blind, placebo-controlled surveys.

In the study by Hammett the growth rate of solely breast-fed infants whose mothers ingested their placenta after childbirth was compared with infants whose mothers did not. The mothers were fed 0.6 g of desiccated placenta three times per day. It was shown that the growth rate of the infants whose mothers engaged in placentophagy was uniformly greater than the growth rate of the infants whose mothers did not (Hammett 1918).

In the survey by Soykova-Pachnerova et al. (Soykova-Pachnerova et al. 1954), 210 mothers with nursing difficulties ingested vacuum frozen-dried placenta. In 86% of cases, the quantity of milk was increased by 20g or more. The study does not offer further detailed information on these results.

A more recent study conducted at Mahidol University in Bangkok, Thailand, investigated the concentration of hormones and nutrients in heated-dried human placenta.

30 placentas from first, singleton term pregnancies were examined, from 15 male and 15 female newborns, all delivered by normal vaginal birth. Placentas of mothers with infections (HIV, Hepatitis, Syphilis) and with medical or obstetric complications were excluded. Placentas were dried for 24 h at a temperature of 80–100° Celsius and then blended into a powder. The analysis data is shown in Table 3, presenting the mean and standard deviation (Phuapradit et al. 2000).

Table 3: **Hormones, minerals, vitamins and macronutrients in dried human placenta (Phuapradit et al. 2000).**

Hormones (ng/g)	**Female**	**Male**
Estradiol	9.35 ± 3.75	8.49 ± 1.27
Progesterone	123.47 ± 39.46	148.27 ± 35.15
Testosterone	19.79 ± 7.95	20.71 ± 4.28
Growth Hormone	3.40 ± 6.76	0.00 ± 0.00
Minerals/Vitamins (mg/kg)	**Female**	**Male**
Sodium	10202.21 ± 320.7	10418.0 ± 704.0
Potassium	8590.9 ± 1020.2	8367.1 ± 574.9
Phosphorus	2807.0 ± 343.2	2800.0 ± 410.6
Calcium	1525.8 ± 1302.8	2287.7 ± 2639.1
Iron	980.0 ± 147.4	1040.0 ± 154.9
Magnesium	373.0 ± 55.6	392.8 ± 59.3
Zinc	47.3 ± 11.4	47.0 ± 4.2
Copper	41.3 ± 35.6	46.3 ± 26.2
Vitamin E (mg/g)	5.9 ± 1.2	50.9 ± 0.9
Macronutrients (%)	**Female**	**Male**
Moisture	6.12 ± 1.14	7.47 ± 1.22
Ash	5.74 ± 0.48	5.75 ± 0.33
Fiber	0.20 ± 0.02	0.15 ± 0.03
Protein	81.62 ± 1.29	80.06 ± 1.58
Fat	1.49 ± 0.36	1.69 ± 0.46

In 2013 Selander et al. conducted a survey, evaluating the self-reported motivations and experiences of 189 women who ingested their placenta. These motivations included mood improvement after childbirth, general health benefits, recommendation by a placentophagy supporter, restoration of hormones/nutrients lost with parturition and improved lactation. The most common method of consuming placenta was the raw or cooked, dehydrated and encapsulated placenta. The positive effects experienced by women ranged from improved mood (40%) to increased energy (26%), improved lactation (15%) and alleviated postpartum bleeding (7%). No negative effects were mentioned by 69% of the women, 20% experienced various negative effects, 7% reported the unpleasant smells of the capsules and 4% headaches (Selander et al. 2013). It has to be taken into consideration that the data of this survey cannot be interpreted as objective evidence. The extent to which these benefits arise cannot be distinguished from the placebo effect. As the author of this study is the owner of a placenta encapsulation service, there is moreover a conflict of interest.

Recently, placentophagy became a topic of interest in the scientific community. A cross-sectional survey by 2016 Schuette et al. found that patients with a history of a self-reported mental health disorder were more likely to be willing to consider placentophagy. Health care providers and patients had heard of placentophagy, but were unsure of its benefits and/or risks (Schuette et al. 2016).

In a randomized, double-blind, placebo-controlled pilot study conducted by Gryder et al. in 2016, the effect of placenta capsule intake on the maternal iron status was investigated. With an iron concentration of 0.664 mg/g per placenta capsule the recommended daily placenta capsule dose (2 capsules taken 3 times daily) only provided 24% of the recommended daily allowance. The postpartum maternal iron status was neither significantly improved nor impaired (Gryder et al. 2016).

Trace minerals in steamed, dehydrated placenta tissue were analyzed in a study by Young et al., which showed that the mean concentrations of potentially harmful elements (arsenic, cadmium, lead, mercury, uranium) were well below established toxicity thresholds (Young et al. 2016b). In another study by Young et al., steamed and dehydrated placenta tissue was analyzed for its hormone content using liquid chromatography tandem-mass spectrometry. 16 different hormones were detected at relatively low concentrations, including cortisone, progesterone, 17-hydroxyprogesterone and estradiol (Young et al. 2016a).

2.9.1 Placentophagy and Depression in Childbed

Depressive disorders are the most common psychiatric manifestations in parturitional women, with a prevalence of 19.2% in the first 3 months after birth. A postpartum depression (PPD) has to be distinguished from "Baby-Blues", which occur during the first 10 days after parturition in 50–80% of women (Hubner-Liebermann et al. 2012). The "Baby" or "Maternal Blues" are a transitory mild depression with tearfulness, often considered as a normal finding after delivery. Causes are physical discomfort, perineal soreness, psychological difficulty at the onset of lactation and hormonal changes (Pitt 1973). In contrast, Bloch has established the following risk factors for post-partum depression: a history of mental illness including PPD, premenstrual

dysphoric disorder and mood symptoms during the third trimester (Bloch et al. 2006).

According to Gurel, the following additional psychosocial risk factors for postpartal low mood are evident: grandmultiparity, short inter-pregnancy interval and low educational level (Gurel und Gurel 2000).

Hendrick summarized other psychosocial stressors that can contribute to the symptom of PPD: a lack of support, marital conflict, unemployment, an unplanned pregnancy, single motherhood and younger age (Hendrick et al. 1998).

The postpartal wellbeing of the mother has an impact on the cognitive and emotional development of the child (Hubner-Liebermann et al. 2012). For both mother and child, it is desirable to prevent women from experiencing depressive episodes after childbirth. Exposure to maternal depression has a direct impact on child health outcomes. Children of depressive mothers are at least two times more likely to have emotional-behavioral difficulties than children of mothers reporting minimal symptoms (Giallo et al. 2015).

3. Aims of this study

As placentophagy gains popularity, interest has become focussed on the exact properties of placental tissue.

The aim of this experimental study was to analyze the concentration of hormones and trace minerals in, and the microbial composition of raw and processed placental tissue.
Based on this data, possible positive and negative effects of human placentophagy were considered.
Further, a literature review with specific reference to human placentophagy was carried out to evaluate the behavior of ingesting the afterbirth.

4. Methods and Materials

4.1 Sources

Pertinent publications were retrieved by searching English-language publications in PubMed, Web of science and Google scholar between 10/2014 and 01/2017, using the key words: “placentophagy”, “placentophagia”, “ingesting placenta”, “eating placenta” and “placenta capsule intake”. The search included experimental, clinical and epidemiological study types from 1917–2014 and comprised reviews, abstracts and articles. In addition, animal studies and studies found on reference lists were evaluated.

English and German reference books have been a useful source of information, in particular “Heilmittel aus Plazenta”, written by the German midwife Cornelia Enning (Enning 2003). Personal communication with placenta encapsulation specialists Lynnea Shrief and Jules Giessing Gourley via E-Mail and Skype was used to adjust the methodology of placenta preparations.

4.2 Financing

This study was made possible through public funding. Funds were raised through the online platform www.experiment.com/placenta, a website for the crowd funding of scientific projects.

Between November 13 and December 13, 2015 an amount of $ 8,355 was raised from 115 backers. This money (less 8 percent platform fee) was directly transferred to Jena University. All the equipment needed for placenta preparation (dehydrator, food processor, steamer pot) and the analysis of all samples by Food GmbH was paid for from this fund.

4.3 Placenta Sample Collection

This study was approved by the ethics committee of the University of Jena. Written informed consent was obtained from all women who gave birth at the obstetric department of the Jena University Clinic and donated their placenta to the Placenta Laboratory for scientific research. Exclusion criteria were C-section, the use of synthetic oxytocin and infection of either the mother or newborn.

Basic data including mothers' age, gravida- and para-status as well as newborn and placenta data were taken.

4.4 Sample Preparation

Nine placentas, all from spontaneous, non-interventional birth were collected at the Obstetric Department of the Jena University Clinic. The placentas were taken immediately after spontaneous birth to the Placenta Lab in clean containers. Within 2 h of placenta birth a swab was taken from both sides (maternal and fetal) of the placenta.

Weight, height and diameter of the placenta were measured and the placenta carefully inspected. Then the placenta was washed under cold running water; blood and blood clots were removed. To replicate the most common method of placenta preparation in a home-based environment, the processing was done under clean but not sterile conditions.

The placenta was cut into three pieces and the umbilical cord was removed.

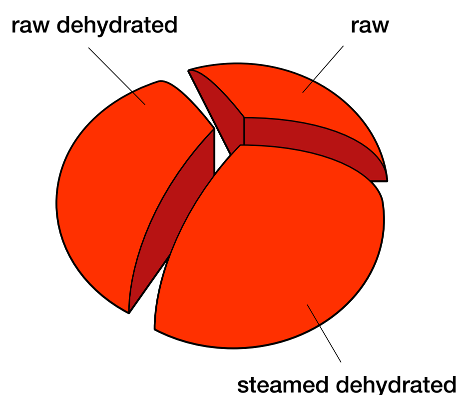

Figure 4: Sample Preparation. Each placenta was cut into three pieces to represent the different hormone concentrations in various placental regions.

One piece ("r" = raw) was homogenized using a meat processor (Moulinex DP800G, Frankfurt am Main, Germany) and samples were taken from the raw homogenized tissue.

Another piece ("s" = steamed) was steamed using a steamer pot with boiling water. Following the preparation method of Traditional Chinese Medicine (TCM), ginger, lemon and hot pepper was added to the water. To avoid variations in the preparation process in our experiments (e.g. different concentrations of volatile oils in fresh ginger), boiling water without added herbs was used for steaming.

The piece was steamed for at least 10 min until the core temperature reached 70 °C and there was no bleeding when pressed.

After the steaming process a sample was taken for microbiological analysis. The steamed piece was cut into 0.5 cm thin slices and placed on backing foil in a dehydrator tray ("s-dhy" = steamed dehydrated).

A third piece of placenta ("r-dhy" = raw dehydrated) was cut into 0.5 cm thin slices and placed on backing foil in a dehydrator tray.
The raw and steamed placenta slices were dehydrated for 8 hours at a temperature of 55°C using a food dehydrator (Stöckli Dörrgerät, Netstal, Switzerland). The heating period of the dehydrator was 20 min, the timer was set for 12 h. The temperature in the upper and lower part of the dehydrator varied during the 8 h dehydration process (Table 4).

Table 4: Variation of temperatures in the bottom and top tray of the dehydrator during 8 h dehydration process.

Sample	Temperature in °C Bottom Tray	Temperature in °C Top Tray
P2	54-58	53-56
P3	54-59	47-56
P4	45-56	49-55
P5	51-59	50-60
P6	50-59	50-53
P7	49-59	49-60
P8	54-58	49-59
P9	51-59	47-56

After 8 hours of dehydration the samples were inspected, and a "snap test" was done to evaluate the complete desiccation of the material. If the material was snap dry, the dehydration process was stopped. If the material was still flexible, the dehydration was resumed for another hour.
During the preparation process the working space was cleaned and disinfected using antibacterial Wipes (Disinfectant Mikrobac forte 0.5 %).
Scalpel, scissors and all utensils made of steel were autoclaved after each complete preparation process.
The samples for microbiological analysis were sent immediately to the Microbiology Department of the Jena University Hospital.

The samples for hormone and trace element analysis were stored in a freezer (–20 °C) until all samples were collected. They were all transferred at the same time after a period of 9 weeks to the laboratory of "Food GmbH, Jena, Germany".

From each placenta a retained sample of steamed dehydrated and raw dehydrated tissue was stored at room temperature and in a fridge (4 °C). After 6 months (± 8 weeks) the samples were analyzed again at the Institute of Medical Microbiology, University of Jena, to evaluate the microbiological growth. A summary of samples taken is displayed in Table 5.

Table 5: Number of placentas used for further analysis.

Sample	Microbiology analysis	Hormone and trace element analysis	Microbiology analysis after 6 months (retained samples)
Swab fetal	7	-	-
Swab maternal	7	-	-
raw "r"	7	6	-
steamed "s"	7	-	-
raw dehydrated "r-dhy"	7	6	7
steamed dehydrated "s-dhy"	7	6	7

Figure 5: Homogenization of raw placental tissue using a meat processor.

Figure 6: 10 min steamed placenta (the pot was covered with a lid during steaming).

Figure 7: Cutting raw and steamed placenta; placing slices on dehydrator tray.

Figure 8: Dehydration of placenta slices.
Raw slices were placed in the bottom tray, steamed slices in the upper tray. The temperature was monitored in both trays.

Figure 9: Dehydrated slices of raw placental tissue were ground and samples taken from the powder.

4.5 Sample Analysis

4.5.1 Microbiologic sample analysis

Samples of raw, steamed, raw dehydrated and steamed dehydrated tissue and swabs of seven placentas were sent to the Institute of Medical Microbiology at the Jena University Hospital for microbiological analysis. Standard routine procedures were used to identify potentially pathogenic bacteria and fungi.

A loop was used to inoculate agar plates with the powder from steamed, dehydrated and ground placental tissue. The placenta samples were streaked onto Columbia sheep blood agar, chocolate agar, Drigalski lactose agar, Schaedler agar, (Oxoid, Thermo Fisher Scientific, Wesel, Germany) using a wet swab. Additionally, for enrichment of microbes brain-heart infusion broth (BHI; BD, Heidelberg, Germany) was inoculated with the powder from steamed, dehydrated and grinded placental tissue.

Cultures were incubated at 37° C at aerobic conditions with 5% CO_2 for blood and chocolate agar for 48 h. Drigalski agar plates were incubated at aerobic conditions for 24 h. Cultures on Schaedler agar were incubated at anaerobic conditions for 96 h. BHI broth was streaked onto blood and chocolate agar after overnight incubation.

These culture media are appropriate to isolate common microbial species of the normal vaginal flora, *Staphylococcus aureus*, hemolytic streptococci, enterococci, Enterobacteriaceae including enteropathogenic species, non-fermenters including *P. aeruginosa, Candida* spp. and filamentous fungi.

Identification of bacteria and fungi was performed using the examination of specific colony morphologies, characteristic growth on differential and selective media, and further species identification with MALDI-TOF mass spectrometry (Vitek MS, bioMerieux, Nürtingen, Germany) if necessary.

Antimicrobial Susceptibility Testing was performed using Vitek 2 and minimal inhibitory concentration interpretation based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria.

4.5.2 Microbiological analysis of retained samples

To replicate the most common methods of storage, a retain sample of r-dhy and s-dhy tissue from every processed placenta was stored at room temperature and in the refrigerator (4° Celsius). After 6 months (± 8 weeks) of storage, samples from raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) placenta were analyzed again for possible further bacterial or fungal growth. The samples were streaked onto Columbia sheep blood agar, chocolate agar, Drigalski lactose agar, Schaedler agar, (Oxoid, Thermo Fisher Scientific) and brain-heart infusion broth (BHI; BD) and incubated as described above. The organisms were identified by VITEK mass spectrometry (MALDI-TOF, bioMerieux).

4.6 Hormones, trace elements and protein content

Hormone concentrations, trace elements and protein contents in samples from raw, raw dehydrated and steamed dehydrated tissue of six placentas were examined in an external laboratory (Food GmbH Jena, Analytik-Consulting, Germany).

Hormone Analysis

The hormone extraction process is based on tissue digestion by an osmotic shock. Placenta tissue samples were treated with a lysis buffer (100 mM NaCl, 50 mM Tris-HCl pH 8.0, 5% Glycerol). After 20 min incubation time samples were centrifuged and the supernatant was transferred to a new reaction tube. Hormone analysis was done immediately afterwards.

The concentrations of CRH, hPL, Oxytocin and ACTH in samples of raw,
steamed dehydrated and raw dehydrated placental tissue were detected using the following ELISA kits: Human OT Elisa Kit (Cusabio Biotech CO., LTD), detection range (8–400µ IU/ml), sensitivity: less than 10 µIU/ml; hPL Elisa (GenWay Biotech, San Diego CA), detection range (0.043–20 mg/l), sensitivity: 0.043 mg/l; CRH Elisa (Cloud Clone Corp., Wuhan, PRC) detection range (12.35–1000 pg/ml), sensitivity: 4.55 pg/ml; ACTH Elisa (Cloud Clone Corp., Wuhan, PRC) detection range (12.35–1000 pg/ml), sensitivity 5.18 pg/ml.

Yeast Estrogen Screening

Yeast Estrogen Screening (YES) is a sensitive tool to identify hormone-active bindings in diluted samples. This bioassay is used to detect endocrine disruptors, for

example in food or drinking water. Genetically modified yeast strains produce specific receptors for steroid hormones, such as the human estrogen receptor or the human gestagen receptor. The activation of the receptor induces an enzyme-mediated discoloration of the samples that is quantified by spectrophotometry. Therefore, the results for the analysis of estrogen and progesterone show the equivalent of substances activating the human receptor for these hormones.

ICP-MS Measurement

The elements As, Cd, Fe, Pb, Se, Hg were determined for all samples by wet digestion with oxidizing reagents (nitric acid, hydrogen peroxide) under increased pressure and temperature. This method is based on DIN EN ISO 17294-2:2017-01 and is typically used for the analysis of food samples. Therefore, it was also used for the placenta tissue samples that were treated in this case study as food samples.

For each element measurement, calibration was done using a blank solution and the diluted ICP multi-element standard solution XXI for MS (Merck). In order to substantiate the results, an internal standard was used for each measurement:

1 µg/ml Rh; preparation: 0.1 ml 1 g/l + 3 ml HNO3 ad 100 ml.

Kjeldahl protein quantification

The quantification of the protein content in a sample was done by standard determination of Kjeldahl-nitrogen. Samples were digested with sulfuric acid in presence of a chemical catalyst (potassium sulphate and copper sulphate). Sodium hydroxide solution was added to the acid digestion mixture to convert ammonium to ammonia gas, followed by boiling and condensation of the ammonia gas in a receiving solution. The amount of ammonia in the receiving solution was detected by titration. The amount of nitrogen in a sample was calculated from the quantified amount of ammonia ions in the receiving solution. The protein content was calculated as the product of the amount of nitrogen and the conversion factor 6.25.

Each measurement was done along with a blank and an internal reference analysis.

4.7 Data Evaluation

Descriptive data analysis was used to characterize the microbial composition of the tissue samples. The absolute number of detected microbial species was used to compare the outcome of microbial composition of the different preparation methods.
The effect of different preparation methods of placenta tissue on its hormone content was evaluated using the Friedman test. This test was used to match the three different preparation methods and hormone concentrations.
The Wilcoxon test was used subsequently to evaluate the relationship between the two different methods. The Wilcoxon test compared two sets of scores that came from the same placenta sample.

5. Results

This study was approved by the ethics committee of the University of Jena. At the Department of Obstetrics of the Jena University Hospital written informed consent was obtained from all women who gave birth and donated their placenta to the placenta laboratory for scientific research. Exclusion criteria were C-section, the use of synthetic oxytocin and infection of either the mother or newborn. All placentas were delivered at term, gestational age ranging from 37+6 to 40+6 weeks. Three women gave birth without any medication, two women had local anesthesia during delivery, two women had other medication (Methyldopa, Butylscopolaminium bromid, Meptazinol hydrochlorid) during delivery.

The first Placenta (P1) was used to test the preparation methods for this study and was removed from the analysis. The samples were labeled P2–P9. One placenta (P7) was excluded from hormone and trace mineral analysis because of synthetic oxytocin administration during delivery, but was included in bacteria testing. P6 also was excluded from hormone analysis for reasons of gender equality (n=3 male and 3 female newborns).

Data from all patients participating in this study were obtained, including newborn and placenta data. The mean weight of the analyzed placentas was 563.7 g, the mean placenta diameter was 18 cm.

Detailed patient information can be found in Table 6.

Table 6: Patient characteristics including newborn and placenta data, (n=8).

	Mean	Range	SD
Age, y	30.26	20–36	4.8
Gravida	2	1–3	0.5
Para	1.9	1–3	0.6
Gestation week	39.8	37+6–40+6	–
Birth weight newborn, g	3496.3	3170–4190	304.9
Head circumference newborn, cm	33.9	31.5–36	1.2
Body length newborn, cm	51.3	48–54	2
Placenta weight, g	563.73	434–709	91.3
Placenta height, cm	2.13	1.5–2.5	0.4
Placenta diameter, cm	18	16–19	1

5.1 Microbiology

The samples were labelled "swab f" for the swab of the fetal side of the placenta, "swab m" for the swab of the maternal side of the placenta, "raw", "steamed", "r-dhy" (raw dehydrated) and "s-dhy" (steamed dehydrated).
To examine the possible microbiological spoiling of dehydrated placenta tissue, retained samples were analyzed after 6 months (Chapter 5.1.1: Retained samples).

Microorganisms, mainly from the vaginal flora, were detected on swab samples and samples from raw, steamed and dehydrated placenta tissue. The absolute number of species detected varied according to the preparation method.

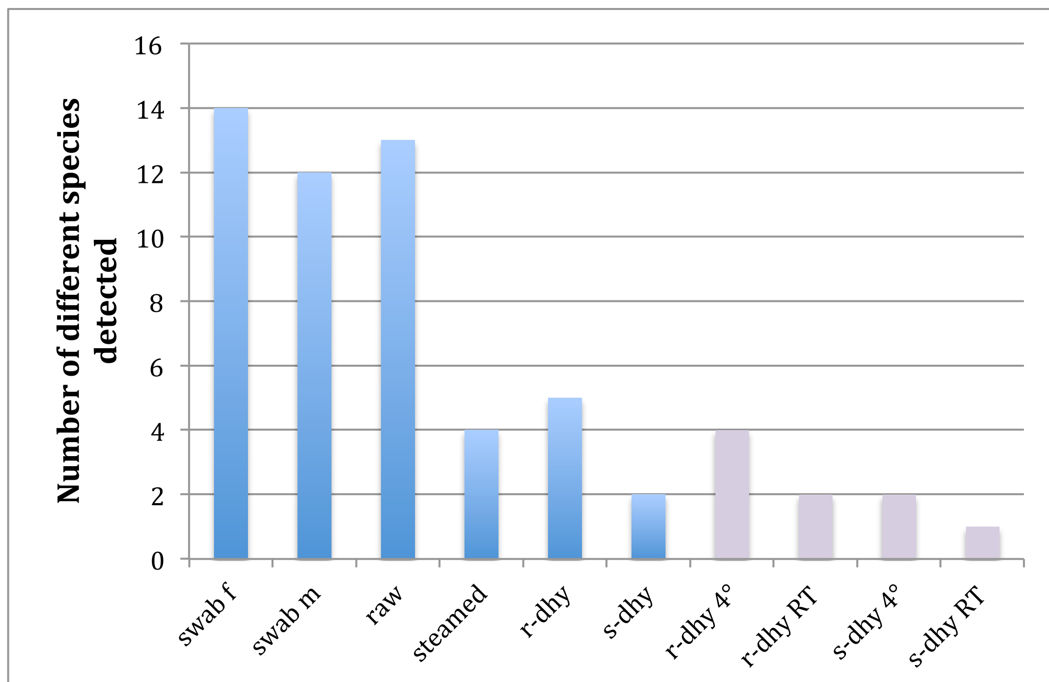

Figure 10: Number of different species detected, n=7.
Blue – Number of different microbial species detected on swab samples (maternal/fetal side of placenta) and on raw, steamed, raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) tissue, **Lilac** – Number of different microbial species detected on raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) tissue of retained samples (after 6 months ± 8 weeks of storage)

The steamed dehydrated tissue showed minimal microbiological growth: in only 1 out of 7 samples were bacteria identified; 6 out of 7 samples showed no bacterial growth after 48 h (Figure 11).

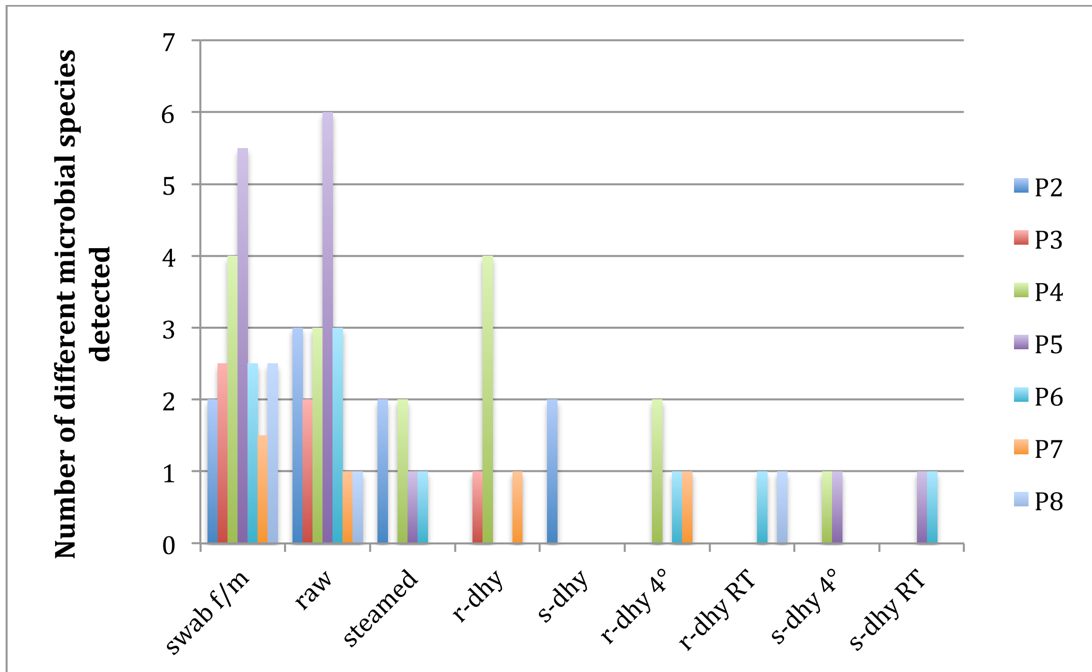

Figure 11: Absolute number of different microbial species detected in each individual placenta (P2-P8).
Samples taken from raw, steamed, raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) tissue and from retained samples, stored at room temperature (RT) and in the fridge (4°).
Swab samples from the fetal and maternal side of the placenta were added and the sum divided by two; a detailed summary with all samples and microbial species detected can be found in the Appendix.

In 4 out of 7 samples of the raw dehydrated tissue, no bacterial growth was shown after 48 h; bacteria of the vaginal flora *(Streptococci, Staphylococci,* Enterobacteriaceae*)* were detected in 3 samples.

The steaming process diminished the number of bacteria species detected on placenta tissue. A piece of placenta was steamed for at least 10 min until the core temperature reached 70 °C. The absolute number of different microbes detected on all samples of raw placenta tissue was reduced from a total of 13 to only 4 different microbes on all steamed samples (Figure 10).

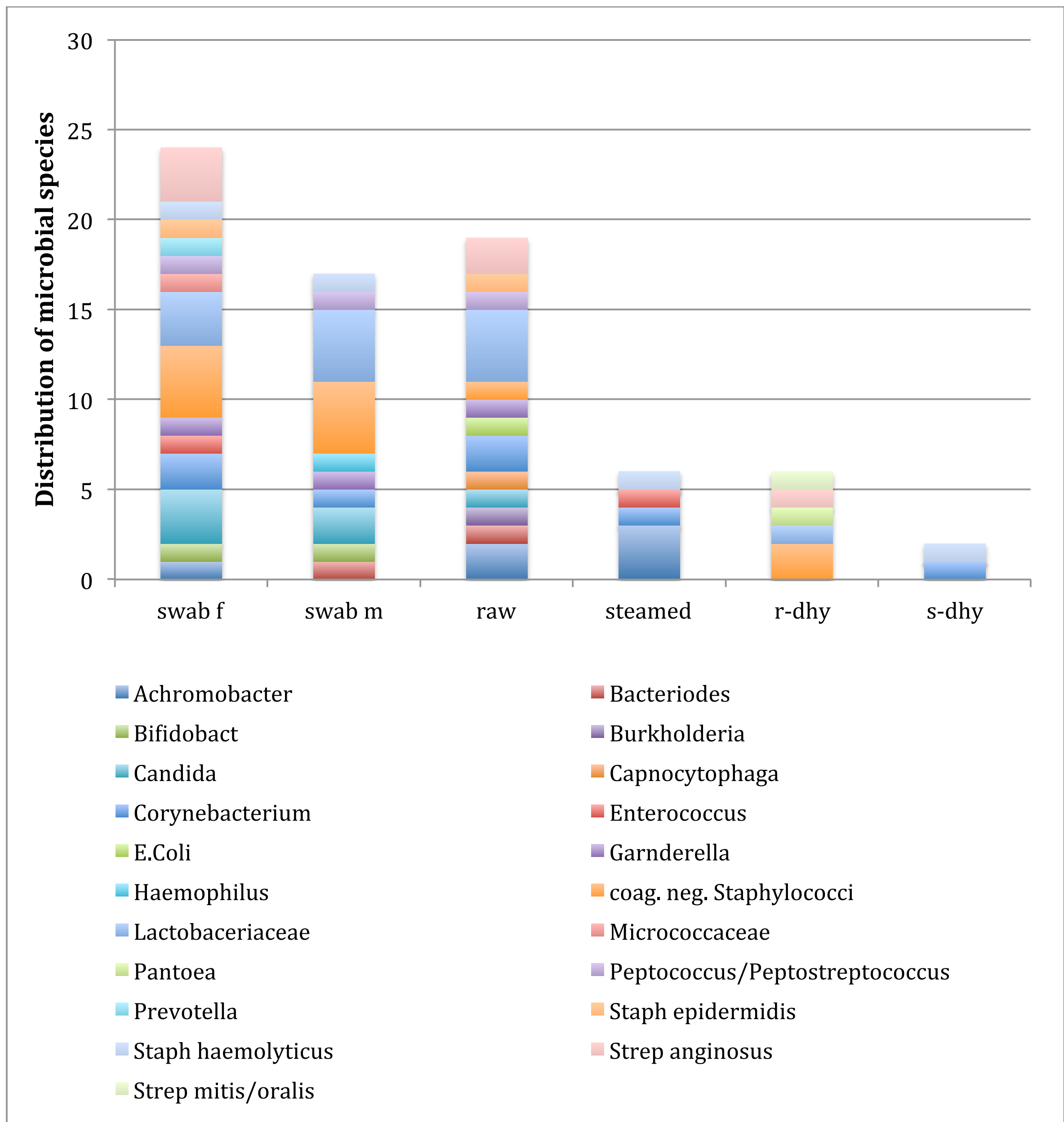

Figure 12: **Distribution of microbial species detected on all analyzed placenta tissue samples (n=7).**
Samples analyzed from swab samples of the fetal (swab f) and maternal (swab m) side of the placenta, and from raw, steamed, raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) tissue. A detailed summary with all samples and microbial species detected can be found in the Appendix.

A total of 14 different species were detected on swab samples from the fetal side of the placenta; a total of 12 different species were detected on the maternal side. A cross-contamination of bacteria from the maternal and fetal sides is probable.

The raw, homogenized placenta tissue was predominantly populated by bacteria of the vaginal and skin flora. The following organisms were identified: Lactobacillaceae, *Corynebacterium* spp, *Staphylococcus* spp, *Streptococcus* spp,

Bacteriodes spp, *Peptostreptococcus* spp, *E. Coli*, *Gardnerella vaginalis*, *Burkholderia gladioli*, *Achromobacter* spp, *Capnocytophaga* spp, *Candida albicans*.

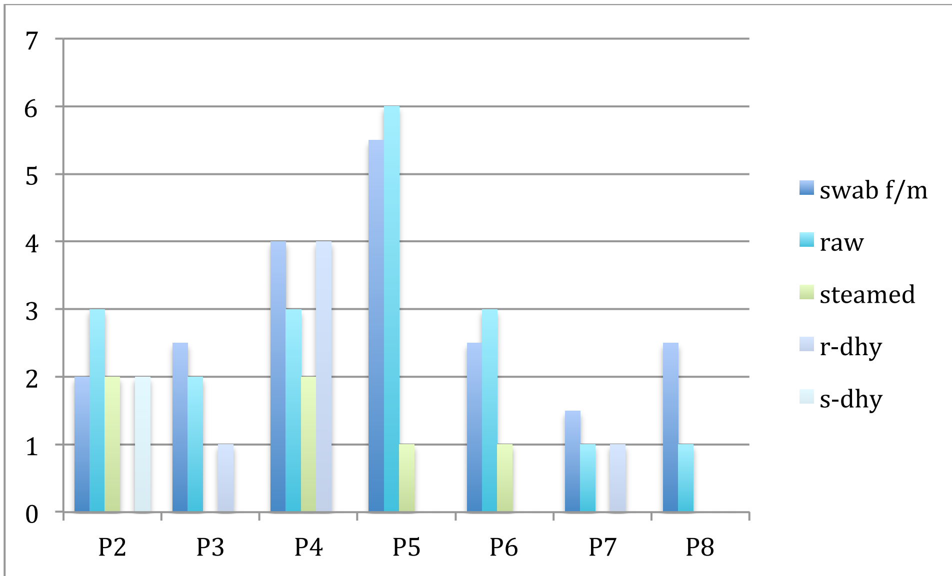

Figure 13: Absolute number of different microbial species detected on each placenta (P2-P8).
Swab samples from the female and maternal side of the placenta (swab f/m) were added and the sum divided by two. The absolute number of different microbial species detected in raw, steamed, raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) tissue is shown.

Raw dehydrated tissue of samples with relatively numerous microbial species detected on raw tissue (P5, P6) showed no bacterial growth after dehydration (Figure 13).

5.1.1 Retained Samples

Women who consume their placenta in capsule form often store the processed placenta tissue for weeks or months. To evaluate the microbiological composition of the dehydreated, pulverized placental tissue, retained samples of every processed placenta in this study were stored for 6 months (± 8 weeks). To replicate the most common method of storage, a retained sample of steamed dehydrated and raw dehydrated tissue from every processed placenta was stored at room temperature and in the fridge (4° Celsius). In 8 out of 28 samples bacterial growth was verified (see Appendix for species details).

The following bacteria were identified:

- *Lactobacillaceae*
- *Coagulase negative Staphylococci*
- *Propionibacterium* spp
- *Bacillus* spp
- *Paenibacillus macerans*
- *Roseomonas mucosa*

5.2 Hormone Analysis

Hormones were analyzed in the following preparation samples of placenta tissue:
raw (r),
raw dehydrated (r-dhy) and
steamed dehydrated (s-dhy) placental tissue.

The median water content of samples from raw placenta tissue in this study was 85%. In order to compare the concentration of hormones in raw and dehydrated tissue with respect to the water content of raw tissue, the concentration of hormones found in raw tissue were converted to dry matter values.

The following formula was used to calculate the relative water content of raw placental tissue:

$$\frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} = \text{realtive water content of raw placenta tissue}$$

In order to calculate the theoretical hormone concentration in dried raw placenta tissue the following formula was used:

$$\frac{\text{Hormone concentration x in raw placenta}}{(1 - \text{relative water content})} = \text{theoretical hormone x concentration}$$

5.2.1 Concentrations of Corticotropin releasing hormone (CRH)

An ELISA Test was performed in order to analyze concentrations of CRH in placental tissue. The highest concentration of CRH was found in raw placenta tissue (median 179.27 ng/g). Proportionately, raw tissue contains a much higher amount of CRH per gram if the water content of placenta tissue is eliminated (median 1206.36 ng/g). The process of dehydration caused a hormone loss of 85.1%, whereas the process of steaming followed by dehydration caused a hormone loss of 95.5%.

Table 7: Tissue CRH concentrations dependent on placenta preparation style: raw (r), raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) in ng/g.

CRH in ng/g	**raw** (wet weight)	**raw** (theoretical dry weight)	**r-dhy**	**s-dhy**
P2	160.94	836.89	215.19	34.64
P3	139.17	1036.04	208.49	61.84
P4	172.91	1671.46	207.99	6.10
P5	185.62	1093.10	263.69	50.72
P8	203.02	1319.63	202.60	99.72
P9	205.61	1347.89	217.51	58.77
Mean	177.88	1217.50	219.25	51.97
SD	23.35	266.70	20.47	28.37
Median	179.27	1206.36	211.84	54.75
25th Percentile	163.93	1050.31	208.12	38.66
75th Percentile	198.67	1340.82	216.93	61.07
Range	139.17 – 205.61	836.89 – 1671.46	202.60 – 263.69	6.10 – 99.72

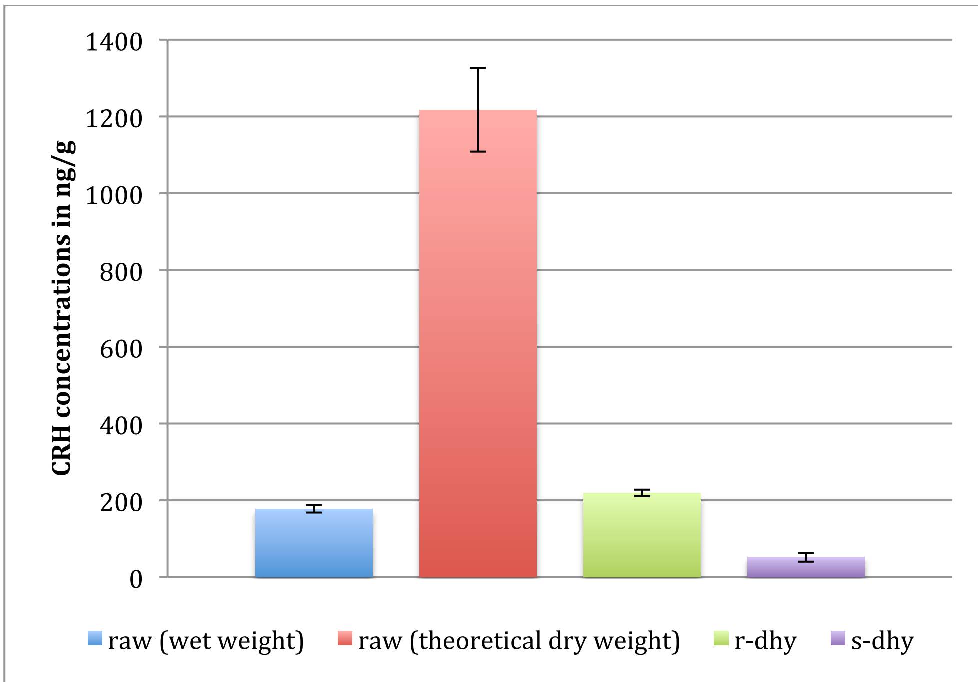

Figure 12: Mean and standard error (n=6) for different placenta preparation styles and their influence on CRH concentrations in ng/g.
The highest concentration of CRH was found in raw placenta (theoretical dry weight).

5.2.2 Concentrations for human placental lactogen (hPL)

An ELISA Test was performed in order to analyze concentrations of hPL in placental tissue. The highest hormone concentration for hPL was found in raw placental tissue (median 17.58 mg/g). The hormone concentration of theoretical dry weight derived from raw placenta is much higher (median 121.89 mg/g). The process of dehydration caused a hormone loss of 87 %, whereas the process of steaming followed by dehydration caused a hormone loss of 99.5 %.

Table 8: Tissue hPL concentrations dependent on placenta preparation style: raw (r), raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) in mg/g. All concentrations below detection limit were defined as half of the value of the detection threshold.

hPL in mg/g	**raw** (wet weight)	**raw** (theoretical dry weight)	**r-dhy**	**s-dhy***
P2	22.30	115.96	31.05	0.63
P3	17.26	128.49	13.28	0.63
P4	14.72	142.29	16.00	0.63
P5	21.47	126.43	12.86	0.63
P8	14.31	93.02	19.83	0.63
P9	17.90	117.34	15.83	0.63
Mean	17.99	120.59	18.14	0.63
SD	3.04	15.06	6.20	0.00
Median	17.58	121.89	15.92	0.63
25th Percentile	15.36	116.31	13.92	0.63
75th Percentile	20.58	127.98	18.87	0.63
Range	14.31 – 22.30	93.02 – 142.29	12.86 – 31.05	0.63 – 0.63

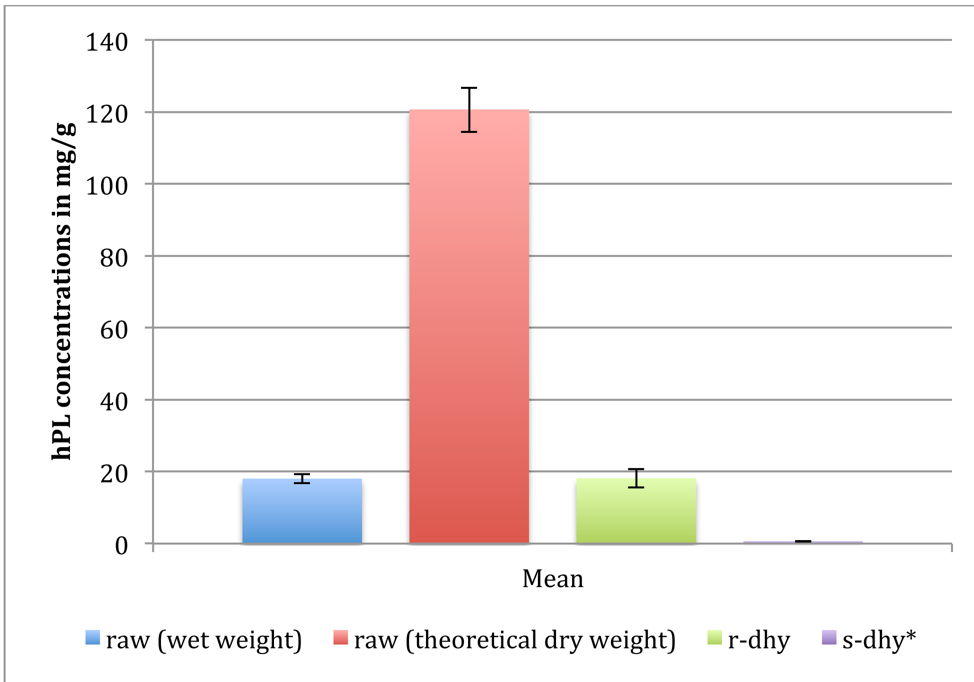

Figure 13: Mean and standard error (n=6) for different placenta preparation styles and their influence on hPL concentrations in mg/g.
The highest concentration of hPL was found in raw placenta (theoretical dry weight).
All concentrations below detection limit were defined as half of the value of the detection threshold.

5.2.3 Concentrations for oxytocin (OT)

An ELISA Test was performed in order to analyze concentrations of OT in placental tissue. The highest hormone concentration for OT was found in raw placental tissue (median 82.58 pg/g). The hormone concentration of theoretical dry weight derived from raw placenta was much higher (median 572.05 pg/g). The process of dehydration caused a hormone loss of 64.6 %, whereas the process of steaming followed by dehydration caused a hormone loss of 89.2 %.

Table 9: Tissue OT concentrations dependent on placenta preparation style: raw (r), raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) in pg/g.

OT in pg/g	**raw** (wet weight)	**raw** (theoretical dry weight)	**r-dhy**	**s-dhy**
P2	105.62	555.89	236.55	61.12
P3	80.80	621.54	167.65	65.67
P4	80.04	800.40	132.92	57.66
P5	84.35	496.18	239.71	62.39
P8	71.57	477.13	646.51	56.07
P9	88.23	588.20	107.38	65.38
Mean	85.10	589.89	255.12	61.38
SD	10.48	106.44	181.74	3.59
Median	82.58	572.05	202.10	61.76
25th Percentile	80.23	511.11	141.60	58.53
75th Percentile	87.26	613.20	238.92	64.63
Range	71.57 – 105.62	477.13 – 800.40	107.38 – 646.51	56.07 – 65.38

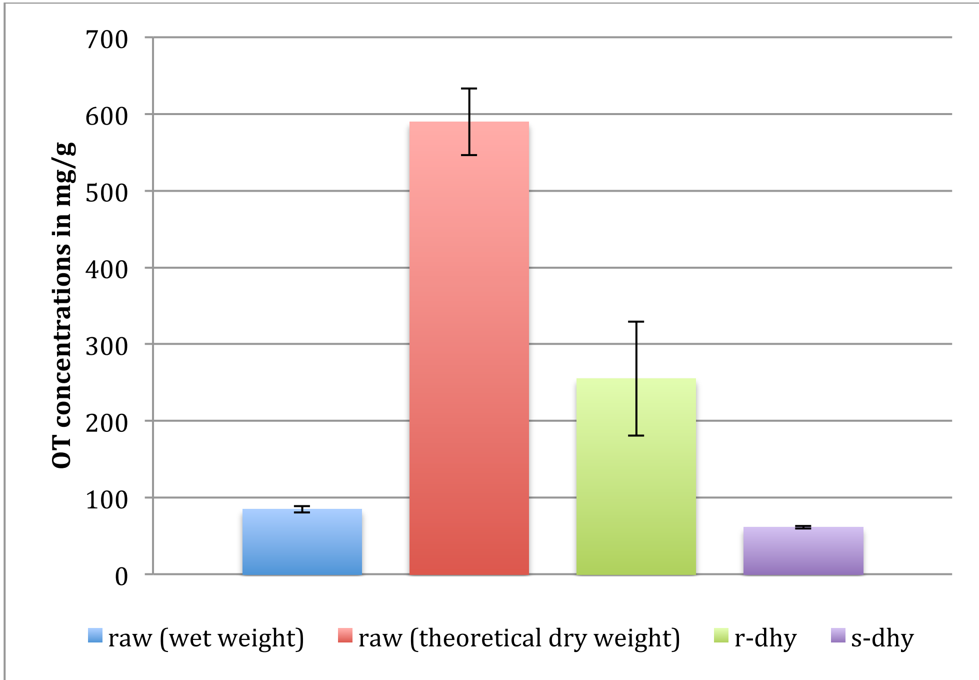

Figure 14: Mean and standard error (n=6) for different placenta preparation styles and their influence on OT concentrations in mg/g.
The highest concentration of OT was found in raw placenta (theoretical dry weight).

5.2.4 Concentrations for adrenocorticotropin hormone (ACTH)

An ELISA Test was performed in order to analyze concentrations of ACTH in placental tissue. The highest hormone concentration for ACTH was found in raw placental tissue (median 2.02 ng/g). The hormone concentration of theoretical dry weight derived from raw placenta was much higher (median 14.26 ng/g). The process of dehydration caused a hormone loss of 91.7 %, whereas the process of steaming followed by dehydration caused a hormone loss of 99.6 %.

Table 10: Tissue ACTH concentrations dependent on placenta preparation style: raw (r), raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) in ng/g. All concentrations below detection limit were defined as half of the value of the detection threshold.

ACTH in ng/g	**raw** (wet weight)	**raw** (theoretical dry weight)	**r-dhy**	**s-dhy ***
P2	1.92	10.11	1.76	0.06
P3	2.04	15.69	1.12	0.06
P4	1.92	19.20	0.94	0.06
P5	2.32	13.65	1.78	0.06
P8	2.00	13.33	1.25	0.15
P9	2.23	14.87	0.85	0.19
Mean	2.07	14.47	1.28	0.10
SD	0.15	2.74	0.37	0.05
Median	2.02	14.26	1.19	0.06
25th Percentile	1.94	13.41	0.99	0.06
75th Percentile	2.18	15.49	1.63	0.13
Range	1.92 – 2.32	10.11 – 19.20	0.85 – 1.78	0.06 – 0.19

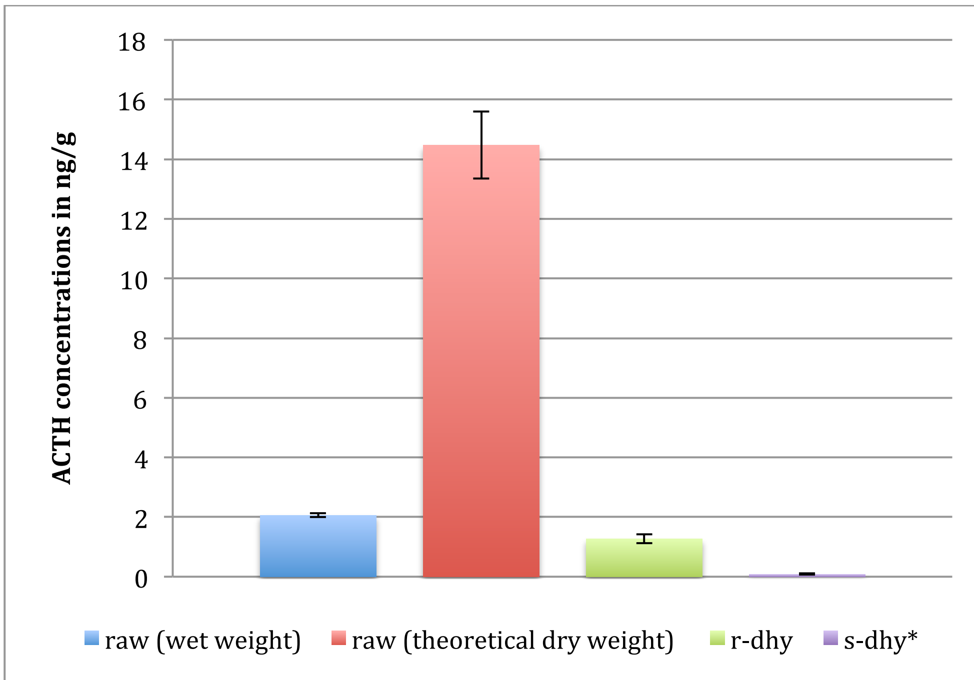

Figure 15: Mean and standard error (n=6) for different placenta preparations and their influence on ACTH concentrations in ng/g.
The highest concentration of ACTH was found in raw placenta (theoretical dry weight). All concentrations below detection limit were defined as half of the value of the detection threshold.

5.2.5 Concentrations for Estrogen Equivalent (EEQ)

A yeast estrogen screening (YES) was performed in order to analyze estrogenically active substances. The highest concentration for EEQ was found in raw placental tissue (median 48.38ng/g). The hormone concentration of theoretical dry weight derived from raw placenta was much higher (median 300.11ng/g). The process of dehydration caused a hormone loss of 97.3%, whereas the process of steaming followed by dehydration caused a hormone loss of 99.2%

Table 11: Tissue EEQ concentrations dependent on placenta preparation style: raw (r), raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) in ng/g.

EEQ in ng/g	**raw** (wet weight)	**raw** (theoretical dry weight)	**r-dhy**	**s-dhy**
P2	55.48	288.50	18.79	3.57
P3	38.84	289.14	6.69	2.68
P4	36.28	350.71	8.57	1.92
P5	51.13	301.10	11.74	2.30
P8	54.32	353.08	7.55	2.73
P9	45.63	299.13	6.01	1.96
Mean	46.95	313.61	9.89	2.53
SD	7.37	27.48	4.38	0.56
Median	48.38	300.11	8.06	2.49
25th Percentile	40.54	291.64	6.91	2.05
75th Percentile	53.52	338.30	10.95	2.72
Range	36.28 - 55.48	288.50 - 535.08	6.01 - 18.79	1.92 - 3.57

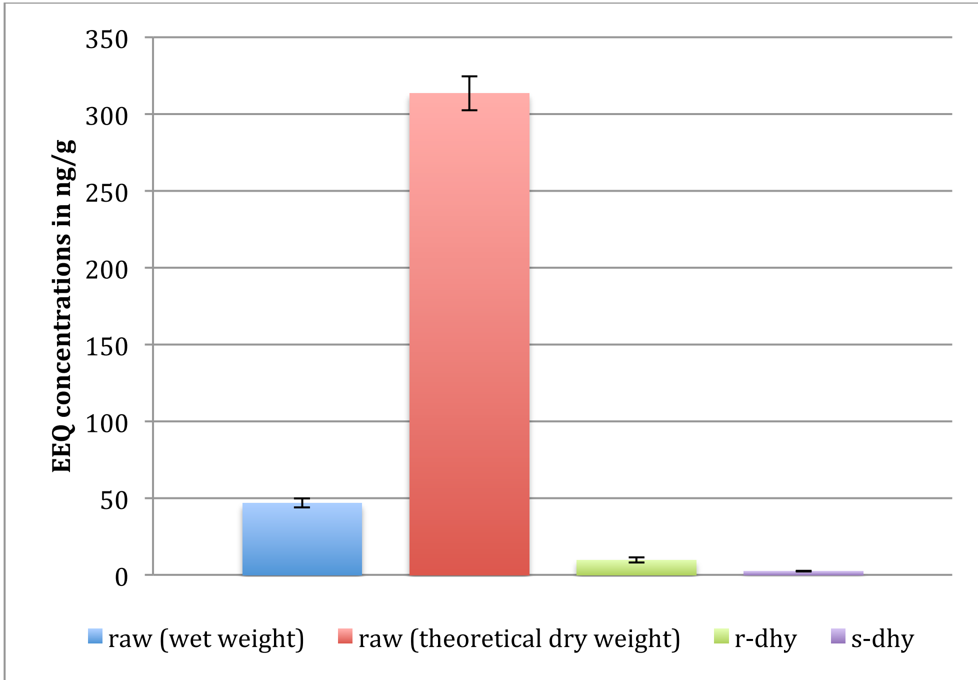

Figure 16: **Mean and standard error (n=6) for different placenta preparation styles and their influence on EEQ concentrations in ng/g.**
The highest concentration of EEQ was found in raw placenta (theoretical dry weight).

5.2.6 Concentrations for progesterone equivalent (PEQ)

A yeast screening was performed to detect gestagenic active substances. The highest hormone concentration for PEQ was found in raw dehydrated placental tissue (median 18.76µg/g). Raw tissue contained a high amount of PEQ if the water content of placenta tissue was extracted (median 14.61µg/g). The process of dehydration caused a hormone increase of 7.3% compared to the concentration in raw tissue. The progesterone equivalent was the only substance that showed an increase in hormone concentration after the preparation process of dehydration. The process of steaming followed by dehydration caused a hormone loss of 97.5%.

Table 12: Tissue PEQ concentrations dependent on placenta preparation style: raw (r), raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) in µg/g.

PEQ in µg/g	raw (wet weight)	**raw** (theoretical dry weight)	**r-dhy**	**s-dhy**
P2	1.85	9.74	9.19	0.46
P3	1.97	15.15	18.93	0.40
P4	1.93	19.30	18.74	0.26
P5	2.39	14.06	10.62	0.38
P8	2.01	13.40	18.78	0.35
P9	2.56	17.07	18.95	0.13
Mean	2.12	14.79	15.87	0.33
SD	0.26	2.99	4.24	0.11
Median	1.99	14.61	18.76	0.37
25th Percentile	1.94	13.56	12.65	0.28
75th Percentile	2.30	16.59	18.89	0.40
Range	1.85 – 2.56	9.74 – 19.30	9.19 – 18.95	0.13 – 0.46

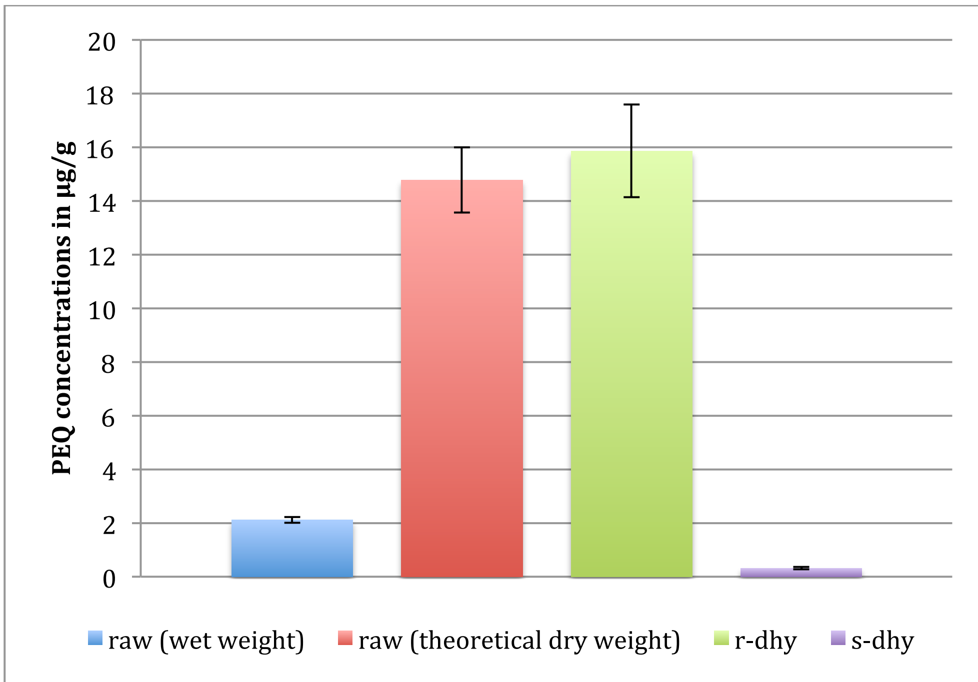

Figure 17: Mean and standard error (n=6) for different placenta preparation styles and their influence on PEQ concentrations in µg/g.
The highest concentration of PEQ was found in raw dehydrated placental tissue.

5.3 Concentration of trace elements

To evaluate the accumulation of potentially toxic elements through placenta preparation, six elements (As, Cd, Fe, Pb, Hg, Se) were analyzed in placenta tissue.

The detection limits for the elements were the following:

Cadmium 0.0007 mg/kg
Lead 0.003 mg/kg
Iron 0.02 mg/kg
Selenium 0.003 mg/kg
Mercury 0.0007 mg/kg

Table 13: Range of trace elements detected in placenta preparations.

	Arsenic µg/kg *	**Cadmium** µg/kg	**Iron** mg/kg	**Lead** µg/kg *	**Selenium** µg/kg	**Mercury** µg/kg *
raw	5.0 – 5.0	2.7 – 9.7	61.0 – 97.0	5.0 – 43.0	138.33 – 150.0	1.0 – 1.0
r-dhy	5.0 – 16.0	19.0 – 28.0	450.0 – 600.0	18.0 – 250.0	700.0 – 850.0	3.0 – 99.0
s-dhy	5.0 – 14.0	14.0 – 23.0	350.0 – 580.0	15.0 – 220.0	720.0 – 1000.0	3.3 – 19.0

Table 14: Concentrations of trace elements (mean and standard deviation).

	Arsenic µg/kg *	**Cadmium** µg/kg	**Iron** mg/kg	**Lead** µg/kg *	**Selenium** µg/kg	**Mercury** µg/kg *
raw	5.0 ± 0.0	4.22 ± 2.5	81.5 ± 11.4	22.5 ± 13.6	138.33 ± 13.4	1.0 ± 0.0
r-dhy	6.8 ± 4.1	24.17 ± 3.6	548.33 ± 48.8	102.17 ± 101.4	826.67 ± 57.3	66.83 ± 31.6
s-dhy	6.5 ± 3.4	19.0 ± 3.6	481.67 ± 75.6	68.33 ± 69.6	851.67 ± 96.9	7.33 ± 5.4

The preparation process caused only minor changes in trace element concentrations, except for the concentrations of iron.

Table 15: Median values for trace element concentrations.

	Arsenic µg/kg *	**Cadmium** µg/kg	**Iron** mg/kg	**Lead** µg/kg *	**Selenium** µg/kg	**Mercury** µg/kg *
raw	5.0	3.0	84.5	26.5	140.0	1.0
r-dhy	5.0	25.0	565.0	41.5	850.0	70.0
s-dhy	5.0	20.0	475.0	42.0	860.0	5.35

* All concentrations below detection limit were defined as half of the value of the detection threshold.

5.4 Protein content

The protein content of placenta tissue from raw, raw dehydrated and steamed dehydrated placental tissue can be compared in Table P1. Due to the moisture reduction, the protein concentration in dehydrated and steamed dehydrated tissue rises.

Table 16: Different protein concentrations dependent on placenta preparation in g/100g.

Total protein in g/100g	raw	r-dhy	s-dhy
P2	12.40	80.90	82.70
P3	12.70	79.80	82.30
P4	11.60	73.60	78.30
P5	12.70	81.50	81.50
P8	13.40	78.80	80.10
P9	14.20	78.70	81.50
Mean	12.83	78.88	81.07
SD	0.81	2.57	1.48
Median	12.70	79.30	81.50
25th percentile	12.48	78.73	80.45
75th percentile	13.23	80.63	82.10

5.5 Statistic analysis of hormone concentration in different placenta tissue preparations

For the statistic analysis of hormone concentrations, data from hormone concentrations in raw tissue (converted to dry matter value), raw dehydrated tissue and steamed dehydrated tissue were compared. Friedman's test was applied to the example data to see if there were differences between the hormone concentrations of the three preparation groups. The SPSS output from running Friedman's test can be observed in Table 17. There was a statistically significant difference in the hormone concentration of every hormone analyzed, which was dependent on the different styles of preparation.

Table 17: Results of Friedman's test: Comparison of hormone concentrations from three preparation groups (raw, steamed dehydrated and raw dehydrated) showed significant differences.

	x^2	*p*
CRH	12.000	0.002
hPL	12.000	0.002
OT	10.333	0.006
ACTH	12.000	0.002
EEQ	12.000	0.002
PEQ	9.000	0.011

A Wilcoxon signed-rank test was applied to compare two sets of data from the same placenta in order to evaluate the three different preparation methods on tissue from one placenta.

Table 18: Comparison of CRH concentrations in r (raw), r-dhy (raw dehydrated) and s-dhy (steamed dehydrated) samples.

CRH			
	r-dhy – raw	s-dhy – raw	s-dhy – r-dhy
p-Value	.028	.028	.028
Based on positive ranks: r-dhy < r, s-dhy < r, s-dhy < r-dhy			

The concentration of CRH in raw tissue was significantly higher than in raw dehydrated and steamed dehydrated tissue.

Table 19: Comparison of hPL concentrations in r (raw), r-dhy (raw dehydrated) and s-dhy (steamed dehydrated) samples.

hPL			
	r-dhy – raw	s-dhy – raw	s-dhy – r-dhy
p-Value	.028	.028	.028
Based on positive ranks; r-dhy < r, s-dhy < r, s-dhy < r-dhy			

The concentration of hPL in raw tissue was significantly higher than in raw dehydrated and steamed dehydrated tissue.

Table 20: Comparison of oxytocin (OT) concentrations in r (raw), r-dhy (raw dehydrated) and s-dhy (steamed dehydrated) samples.

OT			
	r-dhy – raw	s-dhy – raw	s-dhy – r-dhy
p-Value	.046	.028	.028
Based on positive ranks: r-dhy < r, s-dhy < r, s-dhy < r-dhy			

The concentration of OT in raw tissue was significantly higher than in raw dehydrated and steamed dehydrated tissue.

Table 21: Comparison of ACTH concentrations in r (raw), r-dhy (raw dehydrated) and s-dhy (steamed dehydrated) samples.

ACTH			
	r-dhy – raw	s-dhy – raw	s-dhy – r-dhy
p-Value	.028	.028	.028
Based on positive ranks: r-dhy < r, s-dhy < r, s-dhy < r-dhy			

The concentration of ACTH in raw tissue was significantly higher than in raw dehydrated and steamed dehydrated tissue.

Table 22: Comparison of EEQ concentrations in r (raw), r-dhy (raw dehydrated) and s-dhy (steamed dehydrated) samples.

EEQ			
	r-dhy – raw	s-dhy – raw	s-dhy – r-dhy
p-Value	.028	.028	.028
Based on positive ranks: r-dhy < r, s-dhy < r, s-dhy < r-dhy			

The concentration of EEQ in raw tissue was significantly higher than in raw dehydrated and steamed dehydrated tissue.

Table 23: Comparison of PEQ concentrations in r (raw), r-dhy (raw dehydrated) and s-dhy (steamed dehydrated) samples.

PEQ			
	r-dhy – raw	s-dhy –raw	s-dhy – r-dhy
p-Value	.463	.028	.028
Based on positive ranks: r-dhy > r, s-dhy < r, s-dhy < r-dhy			

The findings for progesterone equivalents differed from those for the other hormones. The concentration of PEQ in raw dehydrated tissue was significantly higher than in raw and steamed dehydrated tissue.

In summary, the Wilcoxon signed-rank test showed that all hormone concentrations, except progesterone equivalents, had the significantly highest concentration in raw placenta tissue. All analyzed hormones had a significantly higher concentration in raw dehydrated tissue compared to steamed dehydrated tissue.

6. Discussion

The present study was designed to evaluate the effect of different preparation methods on the properties of placental tissue. Additionally, literature on placentophagy was obtained and carefully reviewed. Based on the findings of experimental results and published data, processed placenta is discussed as a potential supplement for postpartum recovery.

6.1 Possible effects of placentophagy

In a survey conducted by Selander et al. (Selander et al. 2013) women reported the following benefits after placentophagy: improved mood (40%), increased energy (26%), improved lactation (15%) and alleviated postpartum bleeding (7%).
To what extent the subjective feelings of mood stabilization, having "more energy" and enhanced milk supply could be due to placentophagy is discussed in the following.

6.1.1 Improved Mood

In addition to psychosocial factors influencing the perinatal period, drastic hormonal changes occur. Pregnancy is characterized by marked changes in the Hypothalamic-Pituitary-Adrenal-Axis (HPA-Axis) (Yim et al. 2009). During the third trimester of pregnancy placental CRH (pCRH) is detectable in maternal blood, stimulating ACTH release from the anterior pituitary (Sasaki et al. 1984). ACTH causes cortisol release from the maternal adrenal cortex (Smith und Vale 2006). Cortisol levels during pregnancy reach levels found in Cushing´s Syndrome and major melancholic depression (Kammerer et al. 2006). Cortisol again stimulates placental CRH synthesis. During pregnancy, the exponentially increased pCRH reaches levels similar to those of CRH in the hypothalamus during conditions of acute stress (Lowry 1993). These high hormone levels fall immediately after placental birth. It has been argued that the acute withdrawal from these high CRH levels after parturition can explain the occurrence of postpartum depressive disorders (Magiakou et al. 1996).
Yim found a correlation between CRH concentration and PPD. Even if there are many studies with no clear results for hormone substitution therapy as option of

choice for PPD, there is still the hypothesis that the withdrawal of gonadal or neurohormones can influence the mood of certain women. pCRH is produced by the placenta, and plasma CRH concentrations rise exponentially during pregnancy (Sandman et al. 2006). The CRH concentration found in raw placental tissue in this study was 179.27 ng/g. Could it be an option for women to substitute the body´s own CRH with placenta intake to regulate the hormonal situation after birth? The effect of placentophagy on PPD or other mood symptoms still has to be evaluated in clinical studies. Further studies are needed to investigate the bioactivity of CRH after oral ingestion.

As progesterone is the only hormone that is stable to dehydration, our data suggests that it could have an effect on postpartal mood stabilization. According to Bloch et al., marked changes in gonadal steroid levels can produce mood destabilizing effects and the involvement of progesterone in the development of post partum depression has been shown (Bloch et al. 2003). The role of progesterone in the development and treatment of post partum depression must be investigated in more detail.

Even if the reported benefit of stable mood after placentophagy is due to a placebo effect, it nevertheless has an influence on the mother-child relationship. If the intake of processed placenta helps a new mother to cope with the exhausting situation after delivery and in puerperium, and leads – through whatever cause – to a stabilized mood in the mother, there will be advantages for the child as well.

6.1.2 More energy

Anemia is a significant health problem in women of reproductive age and is related to various health problems for the mother and the newborn. Iron deficiency anemia (IDA) with a prevalence of 80% is the most common form (Herold 2009).

The hemoglobin (Hb) threshold used by the WHO to define anemia is <11.0 g/dl in pregnant women and <10.0 g/dl in women post partum (WHO 2008). The prevalence of anemia in pregnant women is 25.1% in Europe and 24.1% in the Americas, compared to a prevalence of 19.0% in non-pregnant women in Europe and 17.8% in non-pregnant women in the Americas (Bruno de Benoist und Erin McLean 2008).

The manifestation of a postpartum anemia is closely associated with the occurrence of IDA in pregnancy (Milman 2008). The short and long term effects of anemia for mother and newborn are wide ranging, as detailed by Milman. Postpartum anemia in women causes decreased physical performance and is associated with an increased

prevalence of tiredness, breathlessness, palpitations, and infections, predominantly of the urinary tract (Milman 2011).

The treatment of IDA with iron supplementation results in an improvement of maternal stress and depression indicators (Beard et al. 2005). Beard concluded that there is a strong relation between iron status and depression, stress and cognitive function. However, Corwin states that early postpartum anemia is a significant risk factor for PPD. Hb levels are expected to rise by day 7 postpartum. When this did not occur, symptoms of depression were soon apparent (Corwin et al. 2003).

Corwin linked early postpartal fatigue with the development of PPD. It was found that women who experience high levels of fatigue on day 14 postpartum scored significantly symptomatic of depression on day 28 postpartum (Corwin et al. 2005).

Therefore anemia has an important effect on the (psychological) wellbeing of the mother and her interaction with the newborn. Assuming that the anemia is caused by iron deficiency, the dietary requirement of iron in pregnant and breast-feeding women is higher compared to that of the normal population.

Two different types of iron are known: nonheme iron, which is present in both plant foods and animal tissues, and heme iron, which comes from hemoglobin and myoglobin in animal source foods (Hurrell und Egli 2010). Taking into account that heme is absorbed intact and more effectively than inorganic iron (Bhagavan 2002), using the iron stores of the placenta can be a good option to meet the postpartal iron demand. According to the U.S. Department of Health and Human Services the recommended iron intake for women of reproductive age (19–51 years) is 18mg/d (Health and Human Services 2015). The dose is increased to 27 mg/d in pregnancy and reduced to 9mg/d in lactating women. A reduction of the daily iron intake in lactating women has to be discussed with respect to the numbers of anemic women during pregnancy and the resulting possibility of postpartum anemia. The logical consequence for women suffering from IDA in the postpartum period would be iron supplementation, preferably with heme iron.

The concentration of iron found in raw, raw dehydrated and steamed dehydrated tissue measured in our study was compared to other published iron levels in processed placenta (Table D1).

Table 24: Published iron concentration in processed placental tissue.
Data compared from samples of raw, raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) tissue, mean and standard deviation in mg/kg.

	raw	r-dhy	s-dhy
Present study	81.5 ± 11.4	548.33 ± 48.8	481.67 ± 75.6
Puaphradit et al. 2000	–	980.0 ± 147.4	–
Young et al. 2016	–	–	664 ± 161.4

When consuming 1g of raw dehydrated placenta powder three times daily, the approximate iron intake is 1.6mg per day. This is 17.8% of the daily intake recommended for lactating women (Recommended Dietary Allowance 9mg/day).
In a randomized, double-blind, placebo-controlled pilot study, Gryder et al. compared the iron status of women ingesting their own encapsulated placenta to those ingesting a dehydrated and encapsulated beef placebo (with a lower iron concentration compared to that of the encapsulated placenta) over a 3-week postpartum period. It was concluded that encapsulated placenta is an inadequate source of supplemental iron in cases of deficiency (Gryder et al. 2016).
The subjective effect of having "more energy" could be linked to the high protein content of dehydrated placenta.
Lactation is a consumptive process for women in childbed and afterwards.
According to Butte et. al, the energy cost of milk production must be added to a women´s basic energy requirement. The energy cost of lactation is met primarily through dietary intake (Butte und King 2005). The recommended dietary protein intake for lactating women is 71g/d (Trumbo et al. 2002).
Dehydrated placenta is a source of the indispensable amino acids Threonine, Valine, Methionine, Leucine, Phenylalanine, Lysine and Tryptophan and other semi-indispensable amino acids. According to Phuapradit (Phuapradit et al. 2000), the protein content of dehydrated placenta is 81.6%. The protein concentration of placental tissue rises with dehydration due to the reduction of moisture.
In this study, a median protein amount of 79.3g/100g was found in dehydrated placental tissue. A daily dose of 3g raw dehydrated placental powder contains approximately 2.4g of protein. It can be concluded that placentophagy has no nutritional benefit, as there are many other sources of proteins in a balanced diet.

6.1.3 Enhanced lactation

During pregnancy the preparation of the human breast for lactation is mediated through an interaction of hormones. The placenta produces progesterone and estrogen in high concentrations, the latter causes high levels of circulating pituitary prolactin (PRL). These hormones, together with a high concentration of human placental lactogen, promote the development of lobular and alveolar breast tissue. The decline in serum progesterone and estradiol concentration after delivery, combined with the elevated prolactin, results in an increase of milk production (Yen et al. 2014). In lactating women postpartum basal PRL levels remain elevated and postpartum amenorrhea persists (Stern et al. 1986).
During breastfeeding, episodes of suckling trigger the quick release of pituitary oxytocin, which induces the flow of milk by contracting the mammary acini and ducts. PRL, hPL and OT have a lactation enhancing effect and placental tissue is a natural source of these hormones. It is stated by Enning that a shortage of oxytocin can be regulated by the intake of the body´s own oxytocin contained in placenta remedies (Enning 2003). As oxytocin triggers uterus contraction, the observed alleviated postpartal bleeding after placentophagy could be additionally due to this hormone activity.
Oxytocin was detected in raw placental tissue with a median concentration of 82.58pg/g. The median concentration of hPL in raw placental tissue was 17.58mg/g. It has to be established whether these hormones are biologically active after oral ingestion. Depending on these findings, ingested placenta could be a lactagogon.

6.2 Metabolism of placental hormones

More research is needed to determine if placental hormones can have a therapeutic effect for postpartum women. The pharmacokinetic properties of these hormones need to be studied. There are different metabolism pathways for protein and steroid hormones. Steroid hormones are well absorbed from aqueous solutions in the human intestine, but absorption and biological activity are not directly related
(Schedl 1965).
The placental protein hormones include larger polypeptides such as prolactin, hPL and growth factors, and complex proteins such as hCG. Peptides are polymers of less than 50 amino acids such as Oxytocin, consisting of 9 amino acids, and CRH, consisting of 41 amino acids.

Current opinion in the field of biochemistry deems it impossible that proteins, ingested and processed by the human digestive system, can retain their structure.
Proteins are broken down from oligopeptides into dipeptides and finally into amino acids by proteases. These enzymes hydrolytically cleave peptide bonds between amino acids of proteins. Usually these peptidases have specific amino acid sequences that they cleave in their substrates (Campbell 2014). This extensive proteolytic cleavage causes the short plasma half-life of peptides that are used therapeutically (Diao und Meibohm 2013). Pepsinogens produced by the stomach are proteolytic enzymes that hydrolyze peptide bonds, with an optimal activity between pH 1.8 and 3.5. The major source of proteases for digestion of ingested proteins is the pancreas, which produces trypsin, chymotrypsin and elastase (enzymes of the serine protease family).
Additionally, small peptides are digested effectively in the small intestine with the help of several other proteases after initial hydrolysis of complex proteins by gastric and pancreatic enzymes (Whitcomb und Lowe 2007).
It may be possible that there is a difference between the digestion of dietary proteins and proteins that are involved in the control of biological processes, such as, for example, protein hormones. Cleavage of peptide bonds may be non-specific as part of degradation during protein catabolism, or highly specific as part of proteolytic cascades and signal transduction events (Verspurten et al. 2009). This leads to the question whether peptide hormones are inactive after proteolysis or if proteolysis can extend the mechanism of action of these hormones.
Usually protein hormones are cleared via endosomal and lysosomal pathways.
It has been shown that hormones are processed through cleavage into hormones with a secondary biological action (Erdmann et al. 2007).
Numerous hormones survive degradation through target cells after endocytosis. Multiple peptides of more than 6–20 residues often survive. The residual peptides could act in unrecognized ways as secondary hormones (Campbell 2014).

6.3 Possible ways for placental hormones to enter the maternal system

Ingesting placenta could be compared to oral drug administration. When pieces of raw placenta are chewed before swallowing, absorption via oral mucosa is possible. The oral mucosa, including buccal, sublingual and gingival mucosa, is well supplied with vascular and lymphatic drainage. Rapid absorption as well as good

bioavailability is known from sublingual drug administration. There will be no first pass metabolism of the liver and no pre-systemic elimination of substances in the gastrointestinal tract (Shojaei 1998). The absorption of peptides through the oral mucosa is described by Senel (Senel et al. 2001). Peptides enter the circulation by passive diffusion, using the intercellular route as a pathway.

Humans do not possess oral proteolytic enzymes, therefore most protein digestion will not take place until passage into the duodenum and small intestine. Thus there is ample potential for peptides and small proteins to be absorbed in the mouth, esophagus or stomach even prior to entry into the small intestine (Campbell 2014).

There have also been interesting studies discussing the intranasal application of peptides (Striepens et al. 2013, Born et al. 2002). Born et al. found that intranasally-administered peptides achieve direct access to the cerebrospinal fluid within 30 minutes, bypassing the bloodstream. It is assumed that peptide molecules travel by the extracellular route, passing through intercellular clefts in the olfactory epithelium and diffuse into the subarachnoid space (Born et al. 2002, Illum 2000).

Neuropeptide concentration in the brain can be pharmacologically increased through intranasal application, as is evident from cerebrospinal fluid studies (Born et al. 2002).

According to these findings there might be a chance for placental protein hormones, from ingested raw placenta, to be absorbed via oral mucosa. This, in theory, cannot happen with encapsulated placenta powder. The capsules that are used for encapsulation are made of either gelatin or hypromellose (HPMC). Both substances are not resistant to gastric acid, but prevent eventual pregastric absorption.

6.4 Preparation styles of placental tissue and their influence on hormone concentrations

Proteins coagulate when they are heated to a higher temperature than body temperature, and denaturation occurs. Heat denaturation results in cleavage of cross-links between peptide chains. Electrophoretic patterns of proteins heated to 50° and 55° Celsius showed no difference compared with unheated proteins. By increasing the temperature to 60° Celsius electrophoretic alteration in protein structure occurs (Tekman und Oner 1966). This leads to the conclusion that steaming placenta tissue

results in the denaturation of protein hormones. Theoretically there is no hormone activity possible after heating the placental tissue above 55° Celsius.

In this study, protein hormones and steroid hormones were identified in placental tissue even after steaming. CRH, hPL, OT, ACTH, EEQ and PEQ were detected in all processed placenta samples.

The results for EEQ and PEQ resemble the equivalent of substances activating the human receptor for these hormones. It can be hypothesized that activation of the human receptor could happen in vivo after placentophagy.

Levels of CRH, hPL, OT and ACTH were quantified using ELISA. The conclusion that these hormones activate the human hormone receptor can not be drawn from this data. The placental tissue was homogenized in all three preparation styles with respect to possible variations of hormone concentrations in different placental regions. The preparation had a huge effect on the median hormone concentration (compare the alteration of hormone concentrations dependent on the preparation process in Table 25).

Table 25: Median hormone concentrations in raw placenta (wet weight) and the hormone loss through processing in %. The median hormone concentration from raw tissue (calculated dry weight), raw dehydrated (r-dhy) tissue and steamed dehydrated (s-dhy) tissue was used to calculate the percentaged hormone loss.

	Median hormone concentration in raw placenta (calculated dry weight)	**Median hormone concentration in r-dhy tissue**	**Hormone loss through dehydration** (in %)	**Median hormone concentration in s-dhy tissue**	**Hormone loss through steaming and dehydration** (in %)
CRH ng/g	1206.36	211.84	–85.1	54.75	–95.5
hPL mg/g	121.89	15.92	–87.0	0.63	–99.5
OT pg/g	572.05	202.10	–64.6	61.76	–89.2
ACTH ng/g	14.26	1.19	–91.7	0.06	–99.6
EEQ ng/g	300.11	8.06	–97.3	2.49	–99.2
PEQ µg/g	14.61	18.76	+7.3	0.37	–97.5

Dehydration caused a hormone loss of 85.1 % up to 97.3 %. Only gestagenic active substances seemed to endure the dehydration process.
The concentration of various hormones in steamed and dehydrated human placenta was evaluated by Young et al. The concentration of many selected hormones was relatively low, and no conclusion about an physiological effect could be made (Young et al. 2016a).
The data in this study shows a hormone concentration reduction from 89.2 % up to 99.6 % in steamed and dehydrated placental tissue. This could explain the low hormone concentrations detected by Young et al.
If placentophagy could cause a physiologic effect to the human endocrine system, it can be hypothesized that the expected impact would be highest from ingesting raw placenta tissue as this has the highest concentration of hormones. In this case, hPL has the highest mean concentration of all detected hormones (121.9 mg/g) and could have a lactagogic effect if it is bioavailable to the maternal organism.
As our data shows, every placenta contains a certain level of hormones and a huge variation in hormone concentration can be observed. A definite conclusion for active agents in placenta tissue cannot be made and will vary with each processed placenta.

It is understandable that women can have an aversion to consuming raw placenta tissue. The esthetically acceptable preparation of raw placenta into placenta pills is a more palatable method of consumption. Taking a pill daily is a more convenient

method of drug application. For this more suitable method of placenta ingestion, a broad loss of hormone concentrations must be accepted.

6.5 Preparation styles of placental tissue and their influence on microbiological composition

The different preparation styles of placental tissue have an apparent impact on its microbial composition. The data from this study cannot be used for statistical analysis but it gives quantitative information about the number of species detected on processed placenta tissue. Comparing the different placenta preparations, raw placenta tissue contains the highest number of different species. A total of 13 different microorganisms, mainly from the vaginal flora, were detected. Steaming reduced the number of species from a total of 13 in raw tissue to only 4 microorganisms on all steamed samples. The process of dehydration also had a clear effect on the reduction of microorganisms and diminished the number of species from 13 in raw tissue to 5 in raw dehydrated tissue (Figure 10). Steamed and dehydrated tissue had the fewest number of microorganisms detected; only 2 different bacteria (*Staph. haemolyticus* and *Corynebacterium spp*) were found.

In three steamed samples (P4, P5, P6) *Achromobacter xylosoxidans*, a bacteria that is found in a wet environment, was detected after steaming. A contamination of the tissue with *Achromobacter xylosoxidans* through steaming or rinsing placentas in the sink of the laboratory could be possible. To avoid artificial contamination, rinsing the placenta with sterilized water before the preparation process is therefore recommended. Sterilized water should also be used for the steaming process.

In one sample of raw dehydrated tissue (P4 r-dhy, see Appendix), growth of Lactobacillaceae and coagulase negative Staphylococci was shown. The retained sample still contained Lactobacillaceae and coagulase negative Staphylococci. Retained placental tissue is not sterile, but storing does not increase microbiological contamination.

Consumers claim their own placenta as lactagogue and therefore store processed placenta over their lactation period. According to the recommendations of the World Health Organization (WHO accessed 05.01.2018) that infants should exclusively be breastfeed for 6 months, samples in the present study were tested for microbial

contamination after 6 months of storage in a setting similar to a home-based environment.

Growth of *Paenbacillus macerans* was identified on four retained samples. This species can produce histamine in preserved foods (Rodriguezjerez et al. 1994). Ingesting high levels of histamine can cause foodborne chemical intoxications. The produced levels of histamine correlate with the level of microorganisms found. In this study, no exzessive accumulation of *Paenbacillus macerans* was identified. Nevertheless, dehydrated placenta is not sterile and the possibility can not be excluded that the encapsulated tissue contains other potential harmful bacteria such as enterotoxine-producing *Staphylococcus aureus* or histamine-producing Enterobacteriaceae. Depending on the vaginal flora of the placenta donor, transmission of resistant microbial species or potentially pathogenic bacteria such as *Enterobacteriaceae*, *Gardnerella* spp or *Candida albicans* to the placenta is possible. A severe infection in the mother is a contraindication for placentophagy.

Microbiological safety regarding the regulation of foodstuffs is achieved for the small sample size of dehydrated placenta tissue in our study. According to the Commission Regulation of the European Union (Journal of the European Union und Official 2005), dehydrated tissue does adhere to the microbiological criteria for foodstuffs. The food safety criteria for "ready to eat" food and "dried dietary foods for special medical purposes" declare the following organisms as unsafe: : *Listeria monocytogenes*, *Salmonella* spp, *Enterobacter sakazakii*, *E. coli* and *Staphylococcus aureus*, which produces staphylococcal enterotoxins.
The dehydrated tissue of our samples did not contain any of the following: *Listeria monocytogenes*, *Salmonella* spp, *Enterobacter sakazakii*, *E. coli* and *S. aureus*.

In this study, a very low number of microbial species was detected in dehydrated and retained dehydrated placental tissue, and microbiological spoilage of retained placenta tissue is improbable. Therefore, we conclude that the infection risk or risk of food poisoning from individual intake of encapsulated placenta is low.

6.6 Preparation styles of placental tissue and their influence on trace element concentrations

Potentially toxic trace elements were analyzed in this study. According to the foodstuff regulations of the Committee of the European Union (EU 2006), the concentrations of cadmium, lead and mercury found in dehydrated placenta tissue are below the maximum level for contaminants in dietary supplements (Table 26). Concentrations of arsenic were below the maximum concentration for foodstuff (EU 2006).

Table 26: Maximum level of contaminants in dietary supplements according to the commission regulation of the European Union.

Element	**Maximum level for contaminant in dietary supplement** (mg/kg)	**Maximum concentration found in raw dehydrated placenta tissue** (mg/kg)	**Maximum concentration found in steamed dehydrated placenta tissue** (mg/kg)
As	No maximum level established	0.016	0.014
Cd	1.0	0.028	0.023
Hg	0.1	0.099	0.019
Pb	3.0	0.25	0.22
Se	No maximum level established	0.85	1.0

These findings are consistent with data from Young et al. (Young et al. 2016b), who detected concentrations of trace elements below toxicity thresholds.

6.7 Limitations of placentophagy research

The measured concentrations of hormones in processed placenta will not provide information on their bioavailability. To obtain information on the possible effects of placentophagy clinical research is needed. Controlled trials with comparisons of serum and saliva parameters from women before, during and after placenta intake need to be conducted. Furthermore, the concentration of substances that can have a physiological effect on the organism varies in each individual placenta. The different types of delivery (vaginal delivery, elective cesarean section, emergency cesarean section) and the presence or absence of labor and maternal and/or fetal distress can have a considerable effect on the placental hormone concentrations at the time of placenta birth.

Another critical point is the possible accumulation of drugs in the placental tissue. Depending on the physicochemical characteristics of drugs taken during pregnancy or administered during delivery, a possible drug passage from the ingested placenta capsule into breast milk has to be considered. There are several drugs with strict indication or contraindication during breast-feeding, such as antihypertensive drugs, antidepressants and antibiotics. For many other drugs no sufficient data on damage to the newborn caused by drug passage into breast milk is available (Hiddemann 2000).
A method for the exact investigation of drug accumulation in placental tissue is the ex vivo placenta perfusion method. It can also be used to predict placental drug transfer (Hutson et al. 2011). As long as there is no data on drug accumulation or excretion into breast milk available, for the safety of mother and newborn only placentas from healthy women after spontaneous vaginal delivery without drug administration should be used for placentophagy. A contraindication for further use of the placenta would be surgical intervention. In that case anesthesia is required and drugs such as benzodiazepines, opioids, inhalative and local anesthetics are used that could be traceable in the placenta.
Until recently, only anecdotal knowledge about side effects of placentophagy existed. A possible adverse reaction to placenta remedies can be galactostasia through excessively enhanced lactation. In addition, there exists no data on the effects of ingesting placental neurohormones such as ACTH that mediate stress reactions through possible cortisone liberation and HPA-axis activation.

The analgesic effect of placentophagy has been studied extensively in rodent models and is the most clearly established benefit supported by systematic investigation
(Kristal et al. 2012). Amniotic fluid ingestion by rodents licking the vaginal area during delivery is probably as important for the studied potentiation of opioid effects as the ingestion of the placenta. Ingesting amniotic fluid is not practicable for humans.
The analgesic effect of placentophagy in rats is limited to additional opioid-administration (Kristal 1991). Acute pain management for women after spontaneous delivery does not include opioid-administration (Spies 2013). Presumably, women would not experience analgesic benefit from placentophagy without an additional treatment with opioids.
According to Heussner et al. there is a relevant species-specific difference in placental steroid metabolism. In contrast to human placenta, CRH is not detected in

rat placenta (Heussner et al. 2016), and no model exists to evaluate postpartum depression in rats. Conclusions from research on placentophagy in rodent models must be treated with caution, as the human placenta and the rodent yolk sac placenta are not identical.

This study evaluates the impact of steaming and dehydration on the hormone and trace element concentration of human placental tissue as well as its microbial composition. The findings from this study need to be confirmed through a repeated study with a larger sample size. Randomized, double-blind, placebo-controlled studies are necessary to determine possible physiologic influences of placentophagy on the human hormone system, and to evaluate the potential use of individual placenta remedies as a postpartum treatment.

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8. Appendix

Table Microbiology

Microorganisms isolated from samples of raw, raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) placental tissue,
⃠ = no growth after 48 hours, same species or groups of microorganisms are colored identically

Paper

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Ehrenwörtliche Erklärung

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Ort, Datum   Unterschrift des Verfassers

	Swab Fetal	Swab Maternal	raw	steamed	raw dehydrated	steamed dehydrated	r-dhy retained Sample (4° Celsius)	r-dhy retained sample (room temperature)	s-dhy retained Sample (4° Celsius)	s-dhy retained sample (room temperature)
P2	Lactobacillaceae Staph. haemolyticus	Lactobacillaceae Staph. haemolyticus	Lactobacillaceae Corynebacterium spp Burkholderia gladioli	Staph. haemolyticus Corynebacterium spp	⦸	Staph. haemolyticus Corynebacterium spp	⦸	⦸	⦸	⦸
P3	coag. neg. Staph Strep. anginosus	coag. neg. Staph Gardnerella vaginalis	Escherichia coli Gardnerella vaginalis	⦸	Pantoea agglomerans	⦸	⦸	⦸	⦸	⦸
P4	Lactobacillaceae coag. neg. Staph Strep. anginosus C. albicans Micrococcaceae Bifidobacterium ssp. Prevotella spp.	Lactobacillaceae Staph. epidermidis Bifidobacterium ssp.	Lactobacillaceae Staph. epidermidis Strep. anginosus	Enterococcus faecalis Achromomobacter x.	Lactobacillaceae coag. neg. Staph Strep. anginosus Strep. mitis/oralis	⦸	Lactobacillaceae coag. neg. Staph Proprionibacteria	⦸	Bacillus spp.	⦸
P5	coag. neg. Staph Strep. anginosus Achromomobacter x. Corynebacterium spp. Gardnerella vaginalis Peptococcaceae	coag. neg. Staph Strep. anginosus Corynebacterium spp. Haemophilus parainfluenzae Bacteroides thetaiotaomicron Peptostreptococcus	Lactobacillaceae Strep. anginosus Achromomobacter x. Bacteroides spp. Capnocytophaga spp. Peptostreptococcus	Achromomobacter x.	⦸	⦸	⦸	⦸	Paenibacillus macerans	Paenibacillus macerans
P6	Lactobacillaceae Staph. epidermidis C. albicans	Lactobacillaceae Staph. epidermidis C. albicans	Lactobacillaceae C. albicans Achromomobacter x.	Achromomobacter x.	⦸	⦸	Roseomonas mucosa	Paenibacillus macerans	⦸	Paenibacillus macerans
P7	coag. neg. Staph Corynebacterium spp.	coag. neg. Staph	Corynebacterium spp.	⦸	coag. neg. Staph	⦸	coag. neg. Staph	⦸	⦸	⦸
P8	Enterococcus faecalis C. albicans	Lactobacillaceae coag. neg. Staph C. albicans	coag. neg. Staph	⦸	⦸	⦸	⦸	coag. neg. Staph	⦸	⦸
P9	-	-	-	-	-	-	-	-	-	-
Number of species	14	12	13	4	5	2	4	2	2	1

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Human placentophagy: Effects of dehydration and steaming on hormones, metals and bacteria in placental tissue

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A B S T R A C T

Introduction: Human maternal placentophagy, the behavior of ingesting the own raw or processed placenta postpartum, is a growing trend by women of western societies. This study aims to identify the impact of dehydration and steaming on hormone and trace element concentration as well as microbial contamination of placental tissue.

Methods: A total of nine placentas have been processed: six were studied for hormone and trace element concentrations; eight were studied for microbial contamination.

The concentrations of CRH, hPL, oxytocin and ACTH in samples of raw, steamed dehydrated and raw dehydrated placental tissue were detected using ELISA. A yeast bioassay was performed in order to detect estrogen equivalent (EEQ) and gestagen equivalent (PEQ) active substances. Elements (As, Cd, Fe, Pb, Se, Hg) were analyzed using ICP-MS. Isolated colonies from tissue and placenta swab samples were identified using Vitek MS.

Results: Following mean hormone concentrations were detected in raw placental tissue:

CRH (177.88 ng/g), hPL (17.99 mg/g), oxytocin (85.10 pg/g), ACTH (2.07 ng/g), estrogen equivalent active substances (46.95 ng/g) and gestagen equivalent active substances (2.12 µg/g). All hormones were sensitive to processing with a significant concentration reduction through steaming and dehydration.

Microorganisms mainly from the vaginal flora were detected on placenta swab samples and samples from raw, steamed, dehydrated and steamed dehydrated tissue and mostly disappeared after dehydration. According to regulations of the European Union the concentrations of potentially toxic elements (As, Cd, Hg, Pb) were below the toxicity threshold for foodstuffs.

Conclusion: The commonly used protocols for preparation of placenta for its individual oral ingestion reduce hormone concentrations and bacterial contamination.

1. Introduction

A growing trend of women in Western societies consuming their own placenta after delivery, referred to as human maternal placentophagy, has developed during the last decades [1]. Purported benefits such as enhanced lactation, improved mood in puerperium, accelerated recovery after birth and the subjective feeling of having more energy are claimed from placentophagy supporters [2,3].

A common method of ingesting placenta today is through encapsulation of dehydrated, pulverized placental tissue [3]. This encapsulation method originates from traditional practices and resembles the most practical procedure of remedy preparation in a home-based environment.

Placental tissue at term shows intensive endocrine activity [4–7]. Human placental lactogen (hPL) has a potent lactogenic effect and is produced by placental tissue with an extremely high secretion rate of about 1–3 g per day at the end of pregnancy [7]. Oxytocin (OT) initiates and sustains milk ejection in response to suckling during lactation [8], reduces postpartum bleeding through myometrial contraction [9] and is a key mediator of complex emotional and social behavior such as pair bond formation and maternal behavior [10,11]. Oxytocin is produced by several organs including the placenta [12] and its gene expression can be found in in the decidua, the amnion and the chorion [6,13]. Ambiguous data exists on the role of placental corticotropin-releasing

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hormone (pCRH) in the prediction and treatment of postpartum depression (PPD) [14,15]. During pregnancy, exponentially increased pCRH reaches levels similar to those of CRH in the hypothalamus during conditions of acute stress [16]. These high hormone levels fall immediately after placental expulsion. It has been argued that the acute withdrawal from these high CRH levels after parturition can explain the occurrence of postpartum depressive disorders [17,18].

Only a small number of reviews [1,19–21] and studies [22–27] examining the effect of placentophagy on humans exist and yet the bioavailability of hormones and their possible physiologic effects remain unclear. Current research on placentophagy aims to gain deeper insight into hormone composition and trace element concentration of placental tissue after encapsulation [28,29]. This exploratory study aims to examine the effect of three different preparation methods and the impact of steaming/dehydration on properties of human placental tissue.

2. Material and methods

2.1. Placenta donors

This study was approved by the ethics committee of the University of Jena. At the Department of Obstetrics of the Jena University Hospital written informed consent was obtained from all women who gave birth and donated their placenta to the placenta laboratory for scientific research.

Nine placentas, all from spontaneous, non-interventional deliveries were collected. Exclusion criteria were diseases in pregnancy that required medical intervention, C-section, the administration of synthetic oxytocin during delivery and infection of either the mother or newborn. All placentas were delivered at term, gestational age ranging from 37 + 6 to 40 + 6 weeks. Three women gave birth without any medication, two women had local anesthesia during delivery and two women had other medication (Methyldopa, Butylscopolaminium bromide, Meptazinol hydrochloride) during delivery. One placenta was used to test the preparation methods for this study and was excluded from the evaluation. Another placenta was excluded from hormone and trace mineral analysis because of administration of synthetic oxytocin during delivery but was included in microbiological analysis. One male placenta was removed: for hormone analysis 50% male and 50% female placentas have been used (n = 3 per group).

Basic data including the mother's age, gravida- and para-status as well as newborn and placenta data were collected (Table 1).

2.2. Sample collection and preparation

To resemble the most common method of placenta preparation in a home-based environment, the processing was done under clean but not sterile conditions.

In order to evaluate the effect of preparation on microbial contamination and hormone and trace element concentration, three different preparation methods were applied to tissue from each placenta.

Table 1
Participant characteristics including newborn and placenta data, (n = 8).

	Mean	Range	SD
Age, y	30.26	20–36	4.8
Gravida	2	1–3	0.5
Para	1.9	1–3	0.6
Gestation Week	39.8	37 + 6–40 + 6	–
Birth weight Newborn, g	3496.3	3170–4190	304.9
Head Circumference Newborn, cm	33.9	31.5–36	1.2
Body length Newborn, cm	51.3	48–54	2
Placenta weight, g	563.73	434–709	91.3
Placenta height, cm	2.13	1.5–2.5	0.4
Placenta diameter, cm	18	16–19	1

Placentas were taken immediately after birth to the placenta lab in clean containers. Within 2 h a swab was taken from the maternal and fetal side of the placenta. Weight, height and diameter of the placenta were documented and the placenta carefully inspected. Subsequently, the placenta was washed under cold running water and blood and blood clots were removed.

The placenta was cut into three pieces and the umbilical cord was excised.

2.3. "Raw" preparation process

One piece ("r" = raw) was homogenized using a food processor (Moulinex DP800G, Frankfurt am Main, Germany) and samples were taken from the raw homogenized tissue.

2.4. "Steamed" and "steamed dehydrated" preparation process

Another piece ("s" = steamed) was steamed using a steamer pot with boiling water.

The piece was steamed at least 10 min until the core temperature reached 70 °C and there was no bleeding when pressed. After the steaming process a sample was taken for microbiological analysis. The steamed piece was then cut into 0,5 cm thin slices and placed on baking foil in a dehydrator tray ("s-dhy" = steamed dehydrated).

2.5. "Raw dehydrated" preparation process

The third piece of placenta ("r-dhy" = raw dehydrated) was cut into 0,5 cm thin slices and placed on baking foil in a dehydrator tray.

Both raw and steamed placenta slices were dehydrated for 8 h at a temperature of 55 °C using a food dehydrator (Stöckli Dörrgerät, Netstal, Switzerland). The dehydration temperature varied from 45 to 59 °C in the bottom tray and from 47 to 60 °C in the top tray. After 8 h of dehydration the samples were inspected, a "snap test" was done to evaluate the complete desiccation of the tissue. If the tissue was snap dry, the dehydration process was stopped. If the material was still flexible the dehydration was resumed for another hour.

The dehydrated placenta slices were grinded using a food processor (Moulinex DP800G, Frankfurt am Main, Germany) and samples were taken from that pulverized tissue. During the preparation process the working space was cleaned and disinfected using antibacterial Wipes (Disinfectant Mikrobac forte 0,5%). Scalpel, scissors and all utilities made of steal were autoclaved after each whole preparation process.

2.6. Retained samples

To evaluate the microbiological contamination of the dehydreated, pulverized placental tissue, retained samples of every processed placenta were stored for 6 months (± 8 weeks). A retained sample of steamed dehydrated and raw dehydrated tissue from every processed placenta was stored in non-sterile reaction tubes at room temperature and in the refrigerator (4 °C).

3. Sample analysis

3.1. Microbiologic analysis

Samples from raw, steamed, raw dehydrated and steamed dehydrated tissue and swabs of seven placentas were sent to the Institute of Medical Microbiology of the Jena University Hospital for microbiological analysis. Standard routine procedures were used to identify potentially pathogenic bacteria and fungi.

A loop was used to inoculate agar plates with the powder from steamed and dehydrated placental tissue. The placenta samples were

streaked onto Columbia sheep blood agar, chocolate agar, Drigalski lactose agar, Schaedler agar, (Oxoid, Thermo Fisher Scientific) using a wet swab. Additionally, for enrichment of microbes brain-heart infusion broth (BHI; BD, Heidelberg, Germany) was inoculated with the powder from steamed and dehydrated placental tissue.

Cultures were incubated at 37 °C at aerobic conditions with 5% CO_2 for blood and chocolate agar for 48 h. Drigalski agar plates were incubated at aerobic conditions for 24 h.

Cultures on Schaedler agar were incubated at anaerobic conditions for 96 h.

BHI broth was streaked onto blood and chocolate agar after overnight incubation.

These culture media are appropriate to isolate common microbial species of the normal vaginal flora, *Staphylococcus aureus*, hemolytic streptococci, enterococci, Enterobacteriaceae including enteropathogenic species, non-fermenters including *P. aeruginosa, Candida* spp. and filamentous fungi.

Identification of bacteria and fungi was performed using the examination of specific colony morphologies, characteristic growth on differential and selective media, and further species identification with MALDI-TOF mass spectrometry (Vitek MS, bioMerieux, Nürtingen, Germany) if relevant [30].

Antimicrobial Susceptibility Testing was performed using Vitek 2 and minimal inhibitory concentration interpretation according to European Committee on Antimicrobial Susceptibility Testing (EUCAST criteria) [31].

Retained samples were analyzed after storage exactly as described above.

3.2. Hormones, trace elements and protein content

Hormone concentrations, trace elements and protein contents in samples from raw, raw dehydrated and steamed dehydrated tissue of six placentas were examined in an external laboratory (Food GmbH Jena, Analytik-Consulting, Germany).

3.3. Hormone analysis

The hormone extraction process is based on tissue digestion by an osmotic shock. Placenta tissue samples were treated with a lysis buffer (100 mM NaCl, 50 mM Tris-HCl pH 8,0, 5% Glycerol). After 20 min incubation time samples were centrifuged and the supernatant was transferred to a new reaction tube. Hormone analysis was done immediately afterwards.

The concentrations of CRH, hPL, Oxytocin and ACTH in samples of raw, steamed dehydrated and raw dehydrated placental tissue were detected using the following ELISA kits: Human OT Elisa Kit (Cusabio Biotech CO., LTD), Detection Range (8-400μ IU/ml), Sensitivity: less than 10 μIU/ml; hPL Elisa (GenWay Biotech, San Diego CA), Detection Range (0,043-20 mg/l), Sensitivity: 0,043 mg/l; CRH Elisa (Cloud Clone Corp., Wuhan, PRC) Detection Range (12,35–1000 pg/ml), Sensitivity: 4,55 pg/ml; ACTH Elisa (Cloud Clone Corp., Wuhan, PRC) Detection Range (12,35–1000 pg/ml), Sensitivity 5,18 pg/ml.

A yeast bioassay was performed in order to detect active estrogenic and gestagenic substances. The results of the analysis for estrogen and progesterone show the equivalent of substances activating the human receptor for estrogen and progesterone.

In order to compare the concentration of hormones in raw and dehydrated tissue with respect to the water content of raw tissue, the concentration of hormones found in raw tissue was converted to dry matter values (calculated dry weight).

3.4. ICP-MS measurement

The elements As, Cd, Fe, Pb, Se, Hg were determined for all samples by wet digestion with oxidizing reagents (nitric acid, hydrogen peroxide) under increased pressure and temperature. This method is based on DIN EN ISO 17294–2:2017-01 [32] and is typically used for the analysis of food samples. Therefore, it was also used for the placenta tissue samples which were treated in this case like food samples.

For each element measurement, calibration was done using a blank solution and the diluted ICP multi-element standard solution XXI for MS (Merck). In order to substantiate the results, the following internal standard was used for each measurement: 1 μg/ml Rh; preparation: 0.1 ml 1 g/l + 3 ml HNO_3 ad 100 ml.

3.5. Kjeldahl protein quantification

The quantification of the protein content in a sample was done by standard determination of Kjeldahl-nitrogen. Samples were digested with sulfuric acid in presence of a chemical catalyst (potassium sulphate and copper sulphate). Sodium hydroxide solution was added to the acid digestion mixture to convert ammonium to ammonia gas, followed by boiling and condensation of the ammonia gas in a receiving solution. The amount of ammonia in the receiving solution was detected by titration. The amount of nitrogen in a sample was calculated from the quantified amount of ammonia ions in the receiving solution. The protein content was calculated as the product of the amount of nitrogen and the conversion factor 6.25.

Each measurement was done along with a blank and an internal reference analysis.

3.6. Data evaluation

Descriptive data analysis was used to characterize the effect of different preparations on microbial contamination. Friedman's Test and Wilcoxon signed-rank Test were performed to compare the three different preparation methods for samples of each placenta.

4. Results

4.1. Trace elements

To evaluate the of potentially toxic elements through placenta preparation, six elements (As, Cd, Fe, Pb, Hg, Se) were analyzed in placental tissue and its preparations (see Table 2).

The preparation process caused only minor changes in trace element concentrations Dehydration and steaming caused an accumulation of iron and selenium. According to foodstuff regulations of the Committee of the European Union [33] (EU), the concentrations of cadmium, lead and mercury found in dehydrated placenta tissue are below the maximum level for contaminants in dietary supplements. Concentrations of arsenic were below the maximum concentration for foodstuff [33].

The participants of our study reported no special exposure to these toxic elements.

4.2. Protein content

Raw placental tissue contained a median protein amount of 12.7 g/100 g with a range from 11.6 to 14.2 g/100 g. A median protein amount of 79.3 g/100 g was found in raw dehydrated placental tissue with a range from 73.6 to 81.5 g/100 g. Steamed dehydrated placental tissue contained a median protein amount of 81.5 g/100 g with a range from 78.3 to 82.7 g/100 g.

4.3. Microbiology

The absolute number of organisms detected varied according to the preparation method (Fig. 1).

Raw, homogenized placental tissue was predominantly populated by bacteria of the vaginal and skin flora. The following organisms were identified: Lactobacillaceae, *Corynebacterium* spp, *coagulase negative*

Table 2
Median and range of trace elements detected in placenta preparations of raw, raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) tissue in mg/kg and the maximum level for contaminants in dietary supplements and foodstuff according to Foodstuff Regulations of the EU1 in mg/kg, limits of detection (LOD) in mg/kg.

MMMM	Arsenic mg/kg	Cadmium mg/kg	Iron mg/kg	Lead mg/kg	Selenium mg/kg	Mercury mg/kg
raw	< 0.01 (< 0.01)	0.003 (0.0027–0.0097)	84.5 (61.0–97.0)	0.03 (0.01–0.04)	0.14 (0.11–0.15)	< 0.002 (< 0.002)
r-dhy	< 0.01 (< 0.01–0.016)	0.025 (0.019–0.028)	565.0 (450.0–600.0)	0.04 (0.02–0.25)	0.85 (0.70–0.87)	0.007 (0.003–0.0099)
s-dhy	< 0.01 (< 0.01–0.014)	0.02 (0.014–0.023)	475.0 (350.0–580.0)	0.04 (0.02–0.2)	0.86 (0.72–1.0)	0.005 (0.003–0.019)
Max. level	0.1	0.1	No max. level established	3.0	No max. level established	0.1
LOD	0.01	0.002	0.05	0.01	0.01	0.002

1. EU. VERORDNUNG (EG) Nr. 1881/2006 DER KOMMISSION | vom 19. Dezember 2006 | zur Festsetzung der Höchstgehalte für bestimmte Kontaminanten in Lebensmitteln. *Committee of the European Union 2006*.

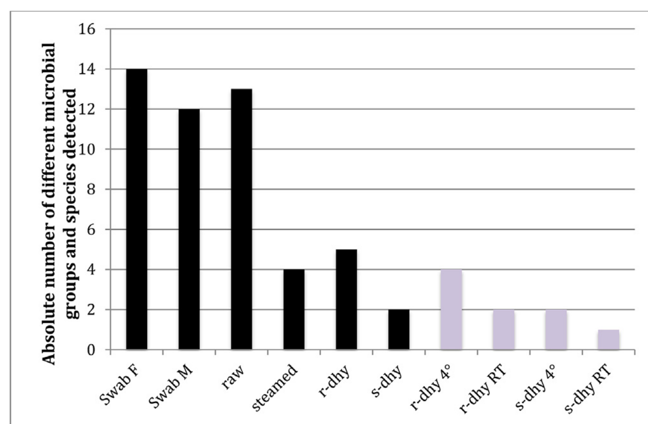

Fig. 1. Absolute number of different microbial groups and species detected and identified by VITEK mass spectrometry on swab and tissue samples.
(Swab F: fetal side of placenta; Swab M: maternal side of placenta).
Black: Samples from raw; steamed; raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) tissue
Grey: retained samples (analyzed after 6 months), RT (stored at room temperature), 4 °C (stored in the refrigerator at 4 °C).

Staphylococcus, *Streptococcus* spp, *Bacteroides* spp, *Peptostreptococcus* spp, *E. coli*, *Gardnerella vaginalis*, *Burkholderia gladioli*, *Achromobacter* spp, *Capnocytophaga* spp and *Candida albicans*.

Raw tissue contained the highest absolute number of microbes detected (13), followed by raw dehydrated tissue (5), steamed tissue (4) and steamed dehydrated tissue (2) (see Table 3 for a detailed list of detected organisms).

Potentially pathogenic organisms *(E. coli, Gardnerella vaginalis)* were detected in raw placental tissue but were absent after dehydration. *C. albicans* was detected on swab samples from 3 placentas, but was absent after dehydration and after steaming.

All samples were negative for group B streptococci.

Growth of *Panbacillus macerans* was identified on four retained samples.

4.4. Hormone content

Hormones were analyzed in different preparation samples:

raw (r), raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) placental tissue.

The median water content of samples of raw placental tissue in this study was 85% with a range from 81 to 90%. In order to compare the concentration of hormones in raw and dehydrated tissue with respect to the water content of raw tissue, the concentration of hormones found in raw tissue were converted to dry matter values (Table 4).

Significant hormone reduction through steaming and dehydration was shown for CRH, hPL, oxytocin, ACTH and estrogen equivalent active substances ($p < 0.05$ for all). The concentration of gestagen equivalent active substances in raw dehydrated tissue was significantly higher than in raw and steamed dehydrated tissue (p 0.028). The Wilcoxon signed-rank test showed that all hormone concentrations, except that of progesterone equivalents, had the significantly highest concentration in raw placenta tissue. All analyzed hormones had a significantly higher concentration in raw dehydrated tissue compared to steamed dehydrated tissue ($p < 0.05$).

The highest mean concentrations for the following hormones were found in raw placental tissue: CRH 177.88 ng/g, hPL 17.99 mg/g, oxytocin 85.10 pg/g, ACTH 2.07 ng/g, estrogen equivalent active substances 46.95 ng/g, gestagen equivalent active substances 2.12 µg/g. Dehydration caused a hormone loss of 85.1–97.3%. Only gestagenic active substances remain stable during the dehydration process with the highest mean concentration found in raw dehydrated tissue (gestagen equivalent active substances 15.87 µg/g).

A dose of 3 g raw dehydrated placental tissue (a daily dosage recommendation according to Enning and Bensky [34,35]) contains approximately the following hormone amounts: 635.4 ng CRH, 47.8 mg hPL, 606.3 pg oxytocin, 3.6 ng ACTH, 24.2 ng estrogen equivalent active substances and 56.3 µg gestagen equivalent active substances.

5. Discussion

The recent study was designed to evaluate the effect of preparation methods on the properties of placental tissue. Based on these findings, processed placenta is discussed as a supplement for postpartum recovery.

The recommended dietary protein intake for lactating women is 71 g/d [36]. According to Phuapradit [37], the protein content of dehydrated placenta is 81.6%. The present study found a mean protein content of 12.8 g/100 g in raw; 78.8 g/100 g in raw dehydrated; and 81.1 g/100 g in steamed dehydrated placental tissue. The protein concentration rises with dehydration due to the reduction of moisture. A dose of 3 g raw dehydrated placental powder contains approximately 2.4 g of protein. It can be concluded that placentophagy has no nutritional benefit, as there are many other sources of proteins in a balanced diet.

In the present study, the mean iron concentration of raw placental tissue is 81.5 mg/kg (SD ± 11.4). Raw dehydrated tissue contains 548.33 mg/kg (SD ± 48.8); this is considerably less iron then detected by Puaphradit [37] et al. in raw dehydrated tissue (980.0 ± 147.4). Steamed dehydrated tissue contains a mean concentration of 481.67 mg/kg (SD ± 75.6) which is nearly the concentration detected by Young [29] et al. (664 ± 161.4).

When consuming 1 g of raw dehydrated placenta powder three

Table 3
Microorganisms isolated from samples of raw, raw dehydrated (r-dhy) and steamed dehydrated (s-dhy) placental tissue,⦸= no growth after 48 h, same species or groups of microorganisms are colored identically.

	Swab Fetal	Swab Maternal	raw	steamed	raw dehydrated	steamed dehydrated	r-dhy retained Sample (4° Celsius)	r-dhy retained sample (room temperature)	s-dhy retained Sample (4° Celsius)	s-dhy retained sample (room temperature)
P2	Lactobacillaceae Staph. haemolyticus	Lactobacillaceae Staph. haemolyticus	Lactobacillaceae Corynebacterium spp Burkholderia gladioli	Staph. haemolyticus Corynebacterium spp	⦸	Staph. haemolyticus Corynebacterium spp	⦸	⦸	⦸	⦸
P3	coag. neg. Staph Strep. anginosus	coag. neg. Staph Gardnerella vaginalis	Escherichia coli Gardnerella vaginalis	⦸	Pantoea agglomerans	⦸	⦸	⦸	⦸	⦸
P4	Lactobacillaceae coag. neg. Staph Strep. anginosus C. albicans Micrococcaceae Bifidobacterium ssp. Prevotella spp.	Lactobacillaceae Staph. epidermidis Bifidobacterium ssp.	Lactobacillaceae Staph. epidermidis Strep. anginosus	Enterococcus faecalis Achromomobacter x.	Lactobacillaceae coag. neg. Staph Strep. anginosus Strep. mitis/oralis	⦸	Lactobacillaceae coag. neg. Staph Proprionibacteria	⦸	Bacillus spp.	⦸
P5	coag. neg. Staph Strep. anginosus Achromomobacter x. Corynebacterium spp. Gardnerella vaginalis Peptococcaceae	coag. neg. Staph Strep. anginosus Corynebacterium spp. Haemophilus parainfluenzae Bacteroides thetaiotaomicron Peptostreptococcus	Lactobacillaceae Strep. anginosus Achromomobacter x. Bacteroides spp. Capnocytophaga spp. Peptostreptococcus	Achromomobacter x.	⦸	⦸	⦸	⦸	Paenibacillus macerans	Paenibacillus macerans
P6	Lactobacillaceae Staph. epidermidis C. albicans	Lactobacillaceae Staph. epidermidis C. albicans	Lactobacillaceae C. albicans Achromomobacter x.	Achromomobacter x.	⦸	⦸	Roseomonas mucosa	Paenibacillus macerans	⦸	Paenibacillus macerans
P7	coag. neg. Staph Corynebacterium spp.	coag. neg. Staph	Corynebacterium spp.	⦸	coag. neg. Staph	⦸	coag. neg. Staph	⦸	⦸	⦸
P8	Enterococcus faecalis C. albicans	Lactobacillaceae coag. neg. Staph C. albicans	coag. neg. Staph	⦸	⦸	⦸	⦸	coag. neg. Staph	⦸	⦸
P9	-	-	-	-	-	-	-	-	-	-
Number of species	14	12	13	4	5	2	4	2	2	1

times daily, the approximate iron intake is 1.6 mg per day. This is 17.8% of the daily intake recommended for lactating women (recommended dietary allowance 9 mg/day [38]). According to Gryder et al. [39] ingestion of steamed, dehydrated placental tissue has no effect on the postpartum iron status.

Potentially toxic elements (As, Cd, Hg, Pb) were below the maximum level for contaminants in dietary supplements and foodstuff regarding regulations of the European Union. These findings are consistent with data from Young et al. [29] and Chang et al. [40] who detected concentrations of trace elements below toxicity thresholds in encapsulated placental tissue. The occurrence of symptoms (e.g. headache) caused by toxic metal accumulation through placenta ingestion discussed by Farr [19] et al. seems negligible, as the detected amounts of potentially toxic trace elements were minimal.

The preparation of placental tissue has a clear effect on the microbial contamination: dehydration causes a drastic germ reduction, steaming followed by dehydration causes an even greater reduction of microbial species. According to the Commission Regulation of the European Union [41], the dehydrated tissue does adhere to the microbiological criteria for foodstuffs.

Consumers claim their own placenta as lactagogue, and therefore, store processed placenta over their lactation period. As according to recommendations of the World Health Organization [42] infants should exclusively be breastfeed for 6 months, samples in the present study were tested for microbial contamination after 6 months of storage in a setting similar to a home based environment. Retained samples showed growth of Panbacillus macerans, a species that can produce histamine in preserved foods [43]. Ingesting high levels of histamine can cause foodborne chemical intoxication. The produced levels of histamine correlate with the level of microorganisms found. In this study, only a low number of colony forming units of Panbacillus macerans was identified. Nevertheless, dehydrated placenta is not sterile and it can not be ruled out that the encapsulated tissue contains other potential harmful bacteria such as enterotoxine producing *Staphylococcus aureus* or histamine producing *Enterobacteriaceae*. Depending on the vaginal flora of the placenta donor, transmission of resistant microbial species

Table 4
Median hormone concentrations in raw placenta (wet weight and calculated dry weight) and the hormone loss through processing in %; the median hormone concentration from raw tissue (calculated dry weight), raw dehydrated (r-dhy) tissue and steamed dehydrated (s-dhy) tissue was used to calculate the percentaged hormone loss.

	Median and Range of hormone concentration in raw placenta (calculated dry weight)	Median and Range of hormone concentration in raw placenta (wet weight)	Median and Range of hormone concentration in r-dhy tissue	Hormone loss through dehydration (in %)	Median and Range of hormone concentration in s-dhy tissue	Hormone loss through steaming and dehydration (in %)
CRH ng/g	1206.36 (836.9–1671.5)	179.27 (139.17–205.61)	211.84 (202.6–263.7)	−85.1	54.75 (6.10–99.72)	−95.5
hPL mg/g	121.89 (93.0–142.3)	17.58 (14.31–22.30)	15.92 (12.9–31.1)	−87.0	< 0.63 (< 0.63)	−99.5
OT pg/g	572.05 (477.1–800.4)	82.58 (71.57–105.62)	202.10 (107.4–646.5)	−64.6	61.76 (56.07–65.38)	−89.2
ACTH ng/g	14.26 (10.1–19.2)	2.02 (1.92–2.32)	1.19 (0.9–1.8)	−91.7	0.06 (0.06–0.19)	−99.6
EEQ ng/g	300.11 (288.5–535.1)	48.38 (36.28–55.48)	8.06 (6.0–18.8)	−97.3	2.49 (1.92–3.57)	−99.2
PEQ µg/g	14.61 (9.74–19.30)	1.99 (1.85–2.56)	18.76 (9.2–19.0)	+28.4	0.37 (0.13–0.46)	−97.5

or potentially pathogenic bacteria such as *Enterobacteriaceae*, *Gardnerella* spp or *Candida albicans* to the placenta is possible.

The highest risk of viral transmission from mother to the fetus occurs during pregnancy and delivery. An infection of mother or newborn is a contraindication for placenta consumption [19]. The mother should have been tested for viral infections during pregnancy (e.g. HIV, HBV, and CMV). If there was a viral infection of the mother, the highest risk for infection results from diaplacentar or perinatal transmission from mother to the newborn.

A single case published by the CDC in 2017 reports a severe newborn infection with group B streptococci and transmission from the mother to the newborn via maternal placentophagy [44]. Questionable in this case is the possible route of infection. The acidic environment of the stomach functions as a physiologic barrier for multiple microorganisms including streptococci. The colonization of the mothers gut with group B streptococci (GBS) as a component of the maternal microbiome with an intrauterine inoculation of the fetus with GBS during pregnancy cannot be excluded [45,46].

In this study, a very small amount of microbial species was detected in dehydrated and retained dehydrated placental tissue and microbiological spoilage of retained placenta tissue is improbable. Therefore we conclude that the infection risk or risk of food poisoning from individual intake of encapsulated placenta is very low.

Although we could not find relevant pathogens that may cause a serious infection after placentophagy, further studies using a larger sample size are needed to evaluate the statistical significance of an infection risk.

The highest concentrations of hormones were found in raw placental tissue. The hormones were all sensitive to processing. The present study found an enormous hormone reduction through steaming and dehydration of placental tissue. It is most likely caused through denaturation of proteins after thermal processing [47]. Dehydration alone caused a minor hormone loss and even increased the concentration of progesterone.

Young et al. evaluated the concentration of various hormones in steamed and dehydrated human placenta: the concentrations of many selected hormones were relatively low and no conclusion regarding an elicit physiological effect could be made [28]. The data in this study shows a hormone concentration reduction from 89.2 up to 99.6% in steamed and dehydrated placental tissue. This could explain the low hormone concentrations detected by Young et al.

Recent publications show only small changes in circulating hormone concentrations and no effect on postpartum maternal mood, bonding, or fatigue after ingestion of steamed or dehydrated placental tissue [22,24].

Effects from ingestion of processed placental tissue are presumably missing after a reduction of up to 99.6% of its hormone content. Focusing on possible hormone interactions from placentophagy, the highest impact would be expected from consuming raw tissue. In this case, hPL has the highest mean concentration of all detected hormones (121.9 mg/g) and may have a lactagogic effect.

But the measured concentrations of hormones in processed placenta does not provide information on the bioavailability of those hormones.

Because of its sensitivity a yeast bioassay was chosen instead of an ELISA for the hormone analysis. The bioassay is able to detect all substances (i.e. also precursor molecules) that induct the human progesterone receptor and the estrogen receptor, respectively. The detection of progesterone equivalents and estrogen equivalents as sum parameters was advantageous for this study. Our data showed that progesterone was the only hormone that remained stable after the dehydration process. The calculated minor increase after dehydration could be explained by fluctuations of the bioassay method. Nevertheless, it was shown that progesterone is still present in a bioavailable form and could be discussed for postpartal mood stabilization. The specific role of progesterone in development and treatment of PPD needs to be investigated in more detail. According to Bloch et al. marked changes in gonadal steroid levels can cause mood destabilizing effects and the involvement of progesterone in the development of PPD has been shown [48].

Farr et al. [19] conclude that there is no professional responsibility for offering placentophagy until reliable evidence on benefits of placentophagy are published. The data provided on improvement of mood and lactation [3] through placentophagy can represent a placebo effect. But nevertheless, if placentophagy subjectively changes the emotional wellbeing of the mother, both mother and newborn can profit from an improved state of health [49].

6. Conclusion

Placental tissue is a natural source of hormones, iron and protein. The exact properties of placental tissue composition vary widely in each individual placenta. Ingesting placental hormones may have a physiologic effect in the postpartum period but no conclusion about the bioactivity of these hormones can be made. The analyzed samples of this study bear a low risk of food poisoning or intoxication but these findings need to be verified with statistical significance in a large sample size.

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